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INVESTIGATION OF EFFECTS OF TEMPERATURE, SALINITY, AND ELECTRODE DESIGN ON THE PERFORMANCE OF AN ELECTROCHEMICAL COLIFORM DETECTOR

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by

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PREFACE

The Federal Water Pollution Control Act Amendments of 1972 charter the Environmental Protection Agency (EPA) to provide a water quality surveillance system for monitoring the quality of navigable waters, ground waters, the contiguous zone, and the ocean. This Act also specifically charges the EPA to utilize the resources of the National Aeronautics and Space Administration (NASA), to the extent practical, to provide such a system. Accordingly, the Director, Surveillance and Analysis Division, EPA Region II, initiated a joint effort with NASA to evaluate the use of NASA's new technique for rapidly detecting coliforms in the coastal waters adjoining the New York Bight. The goal of this effort was to aid EPA in developing technology for an operational system to monitor total and fecal coliforms in waters that lie within the jurisdiction of Region II. The following tasks were identified to meet the program objectives:

TASK 1 - 8-CHANNEL ELECTROCHEMICAL COLIFORM DETECTOR UNITS

Establish a data base using laboratory and field evaluation of the unit in order to compare with standard methods for total coliform measurements. One unit was furnished to the EPA Region II Laboratory, Edison, New Jersey, for a 2-week shipboard evaluation in the New York Bight area. NASA provided personnel to operate the unit and analyze sensor data during the evaluation. EPA personnel performed standard method tests. Continuation of this effort and subsequent tasks delineated in this plan were at the discretion of EPA upon completion of evaluation of the 2-week test results.

TASK 2 - FECAL COLIFORMS MEASUREMENT STUDIES

Determine the optimum detector design for measuring fecal coliforms in saline waters for operational systems. This task was implemented as follows:

(a) Research grants were initiated to study the effects of temperature and salinity on the electrochemical/organism interface performance. EPA provided funding for this task. In addition, EPA implemented this study under the technical guidance of NASA.

(b) NASA performed concurrent in-house studies to aid the grantees in delineating the effects of temperature and salinity on fecal coliforms.

(c) The results of the studies given in Task 2(a) and 2(b) were used to define the optimum sensor design for monitoring fecal coliforms in saline water.

TASK 3 - EVALUATE REMOTE SAMPLING PLATFORMS

Remote sampling platforms to measure total and fecal coliforms in situ were evaluated and information was transmitted to a base station. This evaluation was performed in conjunction with EPA in local waters adjacent to the NASA Langley Research Center. The task was implemented as follows:

(a) Assembled and checked out platforms, sensors, electronics and base station unit.

(b) Performed 1-month test to monitor total and fecal coliforms in water adjacent to the NASA Langley Research Center. Test results were analyzed and documented, and appropriate design modifications implemented, as required.

(c) Additional tests were implemented as specified jointly by EPA and NASA. EPA provided support to deploy and retrieve the platform and personnel to perform standard method tests. Test site location within EPA Region II area was Caven Point, New Jersey. NASA provided the base station equipment in order to communicate with the platform.

TASK 4 - EVALUATE REMOTE SAMPLING PLATFORM IN THE EPA REGION II AREA

NASA refurbished one sampling platform and delivered it to EPA. The platform sensor was modified for demonstration purposes to incorporate the capability to measure fecal coliforms in saline water. This platform was evaluated by EPA and NASA. NASA provided personnel to operate and maintain the unit for a 2-week demonstration test. EPA provided support and personnel to perform standard method tests. Test site location within the New York Bight was determined by EPA subject to the NASA specified design constraints of the platform. NASA provided the base station equipment in order to communicate with the platform. Additional evaluation was at the discretion of EPA with consultation by NASA.

Tasks 1, 3, and 4 were implemented under EPA Interagency Agreement IAG-D6-0930 and are reported in references 2, 3, and 4. This report describes the results of task 2 and is in fulfillment of the Interagency Agreement between NASA Langley Research Center and the Environmental Monitoring and Support Laboratory (EMSL), Las Vegas (EPA-IAG-D7-0053).

The work reported herein was accomplished through two grants: Virginia Institute of Marine Science was awarded a grant to evaluate salinity and temperature as stress factors affecting the enumeration of fecal coliforms by the electrochemical detection of molecular hydrogen. A second grant was awarded the University of Virginia for optimization of waterborne coliform sensor for saline water.

ABSTRACT

This report presents the results of two research programs to determine the optimum detector design for measuring fecal coliforms in saline waters for operational systems. One program was concerned with the effects of temperature and salinity on endpoint detection time and the other, the interaction between electrode configurations and the test organisms.

Test results show that the endpoint response time is related to salinity and seawater temperature; however, these results can be minimized by the correct choice of growth media. Electrode configurations were developed from stainless steel, Parlodion-coated stainless steel and platinum that circumvented problems associated with the commercial redox electrodes.

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SECTION 1

INTRODUCTION

The principal biological indicator of water pollution is the presence of coliforms. In the 14th edition of Standard Methods for the Examination of Water and Wastewater (ref. 1), the approved methods for coliform analysis are the most probable number (MPN) and membrane filtration (MF) tests. Both laboratory tests are laborious and time-consuming, requiring 24-96 hours to complete the MPN test, and 24 hours for the MF test. In view of the increased interest in United States water resources and the ever present threat of fecal contamination, increased emphasis has been placed on the development of automated in situ systems, rather than laboratory methods, to meet the extensive surveillance required to maintain coliform standards.

Recently, Wilkins et al. (ref. 2) described a technique for detecting bacteria based on the time of hydrogen (H_2) evolution. The principle of the electrochemical method is outlined in figure 1. Briefly, it consisted of a reference electrode and a platinum electrode connected to a strip-chart recorder. A typical dose response curve consisted of a lag period, a period of rapid buildup in potential due to hydrogen, and a period of decline in potential (figs. 2 and 3). A linear relationship was established between inoculum size and the time hydrogen was detected (lag period); it was shown that, for example, one bacterial cell could be detected in 7 hours (fig. 4). Further studies indicated that the electrochemical method could also be used to detect fecal coliforms (ref. 3), and the electrodes were readily adaptable to an in situ system. In an extension of these studies, an automated system for the in situ monitoring of coliforms in water was developed to circumvent many of the current problems of enumerating coliforms, viz., sample acquisition, transport, trained laboratory technicians, and the 24 to 96 hours to process a sample. In order to adequately test the in situ concept for monitoring coliforms, a two-phase field evaluation program was conducted. Phase one was conducted in fresh and estuarine waters in the vicinity of the Langley Research Center, Hampton, Virginia, and the second phase was conducted at Caven Point, New Jersey, in conjunction with the Environmental Protection Agency (EPA). Reference 4 describes a pilot model of the in situ monitoring system and presents the results of the field evaluations.

A number of problems were identified during laboratory and field tests of the coliform detector. Among the problems were the effect of salt concentration on detection time and the occasional

instability of the commercial electrodes. For example, when the test system was evaluated using estuarine and freshwater samples, differences in detection times between stock cultures and environmental coliforms were observed. Wilkins and Boykin (ref. 3) suggested that a portion of the coliform population present in the aquatic environment may be stressed with consequent delay in detection times due to exposure of sensitive cells to selective media and elevated incubation temperatures. Data resulting from studies using coliforms exposed to freezing (ref. 32, 37), chlorination (ref. 20), and freshwater environments (ref. 7, 16, and 19) suggested that injured bacterial cells may remain undetected by routine enumeration procedures.

The instability of the electrode systems occasionally manifested itself as either a sudden or gradual departure from the baseline voltage level. The sudden change in voltage levels generally occurs at the beginning of the measurement and gradually reaches a steady-state about 1 hour after inoculation. Both instability problems have been minimized by appropriate designs in the electronics; however, a more reliable electrode would greatly simplify the measurement and cost.

This report presents the results of each task separately. Section 4 contains the results of the evaluation of salinity and temperatures as stress factors. Section 5 contains the results of the electrode sensor investigation.

SECTION 2

CONCLUSIONS

SALINITY AND TEMPERATURE STRESS FACTORS

The ability of an electrochemical detection method to predict viable numbers of fecal coliforms was evaluated under laboratory conditions with respect to seawater adjusted to various salinities and temperatures. The viability of an Escherchia coli isolate as measured by the spread-plate technique utilizing nonselective media was unaffected after 12 weeks exposure at 2°C and 25 ppt salinity. At higher temperatures (15-30°C), both the total decrease in cell numbers as well as the rates of die-off were greater than at 2°C. There was little apparent difference in viability across the temperature range 15-30°C. Viability was observed to be inversely related to salinity over the range 10-30 ppt. Stress was measured using the electrochemical detection method (ECDM), and defined as the difference between the predicted endpoint response time (ER) calculated from a standard curve and the observed ER time. Seawater of higher salinities generally produced greater stress. With respect to temperature, stress was greater at 20°C than at 30°C, while at 2°C stress occurred after a prolonged period of starvation. Delayed ER times were attributed to (1) a reduction in viable cells upon inoculation of starved bacteria into media at 44.5°C and/or (2) an extended lag phase prior to logarithmic growth. Medium A-1 was superior to EC for enumeration of fecal coliforms in estuarine water samples by the ECDM method. ER times occurred sooner and the results were more predictable with the former medium.

STABILITY OF ELECTRODE SYSTEMS

The experiments to date have resulted in the selection of inexpensive materials which can function as a microbe sensing probe for use in culture media. If the probe can be manufactured at a reasonable cost with good reproducibility, the stainless-steel, Parlodion-coated stainless steel system could be used in a variety of applications.

SECTION 3

RECOMMENDATIONS

SALINITY AND TEMPERATURE AS STRESS FACTORS

In order for ECDM to predict coliform levels accurately, it would be advisable to perform additional experiments to determine selective media and temperatures most effective for the recovery of injured coliforms. Our data indicated that Medium A-1 would be preferential to EC at 44.5°C. Moreover, it is essential to have accurate standard curves developed using test media at the same nutrient and selective agent concentrations and temperatures as would be used in obtaining ER data from field samples. It is also imperative that low cell concentrations in the range 10^1 to 10^2 cells/ml be used in developing these standard curves. Standard curves developed during this study varied depending upon the media used, previous cultural history of the organisms, and nutrient and/or selective agent concentration in the media. In addition, there were suggestions that inocula of different volumes, although diluted to the same cell density, could affect ECDM results.

Additional experiments should also be conducted to determine the physiological condition of E. coli (or other bacteria) exposed to the environment under a variety of conditions. The sensitivity of this method for monitoring stress should be examined. A comparison could be made with other techniques capable of detecting sublethal stress, i.e., adenylate charge, enzyme analyses, or growth on selective versus nonselective media.

This investigation has posed additional questions. For example, what role does cryptic growth play in maintaining the apparent viability of starved cultures? Postgate and Hunter (ref. 27) demonstrated that denser populations of E. aerogenes survived longer than sparser ones. Greater stress and viability losses might be observed if starvation experiments were conducted using cell inocula in the range 10^3 to 10^5 cells/ml. Such information would be valuable in further assessing the ECDM as a method of monitoring stress. Viability and stress levels should be compared using cells starved under hypoosmotic versus hyperosmotic conditions to evaluate whether or not the hyperosmotic environment encountered in these studies provided protection to the exposed cells. The degree of leakage of cell constituents under various conditions of salinity should be determined.

STABILITY OF ELECTRODE SYSTEMS

Based upon results of the electrode system investigations, it is recommended that (1) the impedance of the test equipment should be more closely matched to that of the commercial electrodes, and (2) further tests should be conducted on stainless steel and other metals that show promise of functioning as bacteria-sensing probes.

As a corollary to the work on stainless-steel probes, NASA Langley Research Center has conducted tests on platinum wire as one candidate for the bacteria probe. The results of these tests, published in reference 38, show that a linear relationship was established between inoculum size and response time.

SECTION 4

EVALUATION OF SALINITY AND TEMPERATURE AS STRESS FACTORS AFFECTING THE ENUMERATION OF FECAL COLIFORMS BY THE ELECTROCHEMICAL METHOD

EXPERIMENTAL PROCEDURES

Organisms

Fecal coliforms employed in this study were isolated by enrichment in lactose broth at 35°C for 24 hours followed by inoculation into EC broth at 44.5°C for 24 hours. Isolates were obtained from tubes which produced gas at the elevated temperature. All isolates were identified using API-20E (Analytab Products, Inc., Plainview, New York), a miniaturized multiple test system for identification of enteric bacteria. API profile recognition numbers for Escherichia coli isolates were 5 044 552, human fecal isolate; 5 044 562, human urinary tract infection isolate; and 1 144 562, estuarine water isolate obtained from a sample collected from the York River, Gloucester Point, Virginia. Stock cultures were maintained on trypticase soy agar (TSA) at 4°C. During the course of experimentation, isolates were subcultured on TSA three times or less.

Cell Preparation

Inocula for seawater survival experiments were obtained from exponential phase cultures unless otherwise specified. Isolates were grown at 35°C in either trypticase soy broth (TSB) or M-9 minimal medium which consisted of 6g Na_2HPO_4, 3g KH_2PO_4, 0.5g NaCl, 1g NH_4Cl, 0.13g $MgSo_4 \cdot 7H_2O$ and 5 ml glycerol in 1 liter distilled water. The latter two components were sterilized separately and added to the remaining components of minimal medium after autoclaving. The final pH of the minimal medium was 7.0. Bacteria were harvested by centrifugation (1000 x g) at room temperature for 10 minutes and washed three times in phosphate buffered saline (PBS) at pH 7.2. Cells were resuspended to an optical density of 0.2-0.3 (540 nm) in seawater of selected salinities and temperatures.

Survival Experiments

A tenfold dilution of the final cell suspension was made into 90 ml seawater previously equilibrated to the test temperature in 125 ml sterile screw-capped flasks. Seawater at specified

salinities was prepared by diluting aged filtered ocean water (35 ppt) with reverse osmosis/glass distilled water and sterilized by autoclaving. Samples of inoculated seawater were removed at various time intervals for enumeration by spread plating on TSA and determination of endpoint response (ER) in EC and TSB at 44.5°C. Colony counts were made after approximately 24 hours incubation at 35°C. Total exposure time to seawater prior to 0 hour sampling ranged from 2-5 minutes while exposure time to media at 44.5°C prior to dilution for plate counts was less than 1 minute. All dilutions were performed using PBS at room temperature unless otherwise specified.

Endpoint Response (ER) Determination

The experimental design for detecting ER time consisted of a test tube (25 mm by 100 mm) containing two platinum electrodes and 18 ml medium to which 2 ml E. coli seeded seawater was added. Tubes were fitted with a No. 4 rubber stopper containing electrodes of grade A platinum-alloy wire, 24-gauge (0.508 mm) (Englehard Industry, Carteret, New Jersey). Electrodes were inserted into slits made in the stopper, and the stopper bound with wire and/or epoxy to prevent electrode slippage. Electrodes were designed so that the ratio of their lengths below the surface of the medium was 1:4. Sterilization was by flaming over an alcohol lamp. The longer electrode was connected to the negative terminal and the shorter one to the positive terminal of a strip-chart recorder (Model 194, Honeywell Industrial Division, Fort Washington, Pennsylvania, or Model SR-204, Heath Company, Benton Harbor, Michigan). Recorders were set at a chart speed of 10 minutes/inch and operated at 0.5 volt (Honeywell) or 1.0 volts (Heath) full scale. ER times were measured as the time elapsed between challenge and the initial increase in potential difference.

Environmental Samples

Estuarine water samples were collected from the York River at Gloucester Point or from its nearby tributaries which included Yorktown Creek, Sarah Creek, as well as the Northwest and Northeast branches of Sarah Creek. Sampling sites at all localities were subject to varying degrees of pollution from domestic sewage and boats. Water samples were collected in sterile 500 ml Erlenmeyer flasks and processed within 60 minutes. Simultaneous temperature and salinity measurements were made. Parallel fecal coliform enumerations were performed using a five-tube most-probable-numer (MPN) technique (ref. 1) with lactose enrichment at 35°C for 48 hours with subsequent inoculation of positive tubes into EC at 44.5°C for 24 hours, or direct inoculation into Medium A-1 (ref. 5) at 44.5°C for 24 hours. Corresponding ER determinations were made by addition of a 100 ml sample into each of two 250 ml Erlenmeyer flasks containing 100 ml single strength EC or Medium A-1 prewarmed to 44.5°C. Flasks were fitted with rubber stoppers containing the appropriate sterile platinum electrode configuration as specified above.

Standard Curves

Endpoint response times were determined in EC, TSB, and Medium A-1 at 44.5°C for various inocula. Tenfold dilutions of an E. coli suspension (urinary-tract isolate) prepared as for survival experiments were made in 20°C seawater (25 ppt) and inoculated into test media. Exponential cultures were pregrown in either TSB or M-9 media. The relationship between inoculum size and ER time was determined by linear least-squares regression analysis, and confidence belts for the linear regression were established at the 95 percent confidence level (ref. 31).

Data Analysis

During the course of experiments, designed to assess the effect of seawater exposure on ER time, the following data were collected: coliforms/ml seawater, coliforms/ml medium following a tenfold dilution from seawater and ER time in the respective media. Standard curves of ER versus coliforms/ml medium were analyzed by linear-regression techniques. The ER, predicted from the linear-regression line and based on the number of cells/ml medium calculated to have resulted from a tenfold dilution from seawater, was subtracted from the observed ER. This relationship of observed ER minus predicted ER as a function of time in seawater was graphically presented with 95 percent confidence limits for y. Stress was defined as the time delay between observed ER and predicted ER, and was considered significant when this value exceeded the 95 percent confidence limits.

SECTION 5

STABILITY OF ELECTRODE SYSTEMS FOR COLIFORM DETECTORS

ANALYSIS OF COMMERCIAL ELECTRODE FAILURES

The following nine "defective" combination electrodes were provided by NASA to the University of Virginia (UVA). They were reported to have one of two defects--erratic baseline or no response.

Electrode No.	Performance
RSP-1	erratic baseline
RSP-5	erratic baseline
RSP-7	erratic baseline
RSP-8	erratic baseline
RSP-9	erratic baseline
RSP-16	no response
RSP-17	no response
A-1	no response
2	no response

Experiments were performed by comparing at least two electrodes known to function properly with a number of the NASA problematic electrodes. Typical experiments were carried out by preparing a mother culture of trypticase soy broth (TSB) which had been recently inoculated with a low concentration (10^3/ml) of Pseudomonas aeruginosa. The mother culture was then split into 3-ml samples added to a sterile test tube in which the electrode had been placed. The electrodes(up to eight) were then monitored for approximately 24 hours.

All of the electrodes were presterilized by immersion in boiling water for a period of 10 minutes.

The following is a summary of this first set of experiments:

Electrode No.	Performance (NASA)	Performance (UVA)
RSP-1	erratic baseline	OK
RSP-5	erratic baseline	OK
RSP-7	erratic baseline	erratic baseline
RSP-8	erratic baseline	OK
RSP-9	erratic baseline	OK
RSP-16	no response	erratic baseline
RSP-17	no response	erratic baseline
A-1	no response	OK
2	no response	no response

These results indicate that a fundamental disagreement exists between University of Virginia (UVA) procedures/apparatus and those at NASA for at least five of the nine electrodes. For those which displayed "erratic baseline" in UVA experiments, the deviation was much less severe (10-40 mV) than that reported at NASA. Only electrode 2 failed completely, in agreement at NASA and UVA. On examination, the reason for this electrode's failure was apparent in that there was a large leak of KCl at the rate of about 0.2 ml/minute. When arrested, this electrode performed favorably.

In view of the fact that with this one exception, the UVA experiments gave either less severe problems or no problems at all (in five cases), attempts were made to compare the differences in the UVA equipment and that at NASA. Communications with Dr. Judd Wilkins at NASA indicated there were no significant procedural differences in either the microbiology or electrode preparation procedures. However, it was learned recently that there is a fundamental difference in the test equipment: namely, the input impedance of the electronics. The NASA equipment has an input impedance of approximately 10^{14} whereby UVA's is about 10^{10}. Independent of this effort, Wilkins found that by lowering the impedance of the NASA system many, but not all, of the electrode problems were reduced.

UVA has recently proceeded with an examination of RSP-7, 16, and 17, which still did not perform properly. Reducing the concentration of electrolyte in the electrode from saturated KCl to 0.9 percent NaCl diminished the possibility of salt clogging the fritt. This seemed to straighten out the baseline, but also

reduced the overall response about 25 percent. This change from a saturated calomel electrode (SCE) to a normal calomel electrode (NCE) should only change the baseline of the reference electrode from +0.226 to a slightly less positive value according to the Nernst equation. Since the platinum electrode moves in the same direction with micro-organism growth (in the negative direction), the switch from a SCE to a NCE is consistent with a slightly smaller response.

DEVELOPMENT OF A COST-EFFECTIVE DETECTION PROBE

This part of the report describes the series of experiments performed between October 1, 1977, and March 6, 1978, at the Applied Electrochemistry Laboratory, University of Virginia. The goals of these experiments were

To develop an electrochemical probe with the following features:

a. Capable of detecting organism growth in standard culture media.

b. Potentially low enough in cost to be disposable if manufactured in quantity.

Variables investigated:

1. Membrane type and application
2. Electrode materials
3. Media
4. Microbe

All development tests were carried out on an electrode system with one exposed surface and another (reference) electrode coated with a membrane.

Membrane Selection

Experiments 1 through 14: using TSB and P. aeruginosa.- All variables were held constant with the exception of membrane type and application. Membranes studied were agar, 7 percent Parlodion dissolved in acetone, 7 percent Parlodion dissolved in amyl acetate, and a natural fruit membrane. Agar as a membrane was initially investigated by covering an electrode placed in the bottom of a test tube. This test-tube application showed good response to microbe growth; but as a thin surface coating on stainless-steel wire*, it was not conducive to microbial detection when used in a stainless-steel wire--stainless-steel needle coaxial electrode arrangement.

*earlier electrode research by Stoner has shown that 304 stainless steel is a suitable electrode material.

The 7 percent Parlodion (dissolved in acetone) membranes were studied on the basis of applying the material to the stainless-steel wire surface to give maximum response and reliability when used in the coaxial configuration. Membranes were dried at room temperature (approximately 24°C). Note that all membranes were single application to stainless-steel wire.

Seven percent Parlodion (dissolved in amyl acetate) was investigated. It was shown that this membrane, applied as a thin coat and dried at approximately 70° C, was better than all other membrane materials tested.

Experiments 15 through 19: Membrane Optimization Using TSB and P. aeruginosa.- This set of experiments was performed to evaluate the optimum method for producing a uniform membrane (7 percent Parlodion dissolved in amyl acetate) coating on the stainless-steel wire. A single application with only the tip of the stainless-steel wire dipped a second time to cover any sharp edges proved to be more effective than applying either an all-over single coat or double coat of membrane material. The reason was twofold:

1. A double coating increased resistance between wire and needle thus making probe impedance too high.

2. A single coating did not sufficiently cover burrs at the end tip of the wire used in some probes thus causing shorting of that probe.

Pretreatment of the stainless-steel wire surface was also studied. Acid roughening and sanding of the wire indicated no significant increase in detection sensitivity or baseline stability.

At this point, the following assembly protocol was produced. If followed, this assembly procedure gave consistently reproducible results for all systems tested to date. It must be pointed out that these experiments were performed in test tubes with seeded cultures.

Preparation of Disposable Needle Electrodes-

Materials: 18 gauge stainless-steel disposable hypodermic needle; 0.014-inch diameter stainless-steel wire (hypo-grade); Parlodion (cellulose nitrate); amyl acetate; cotton.

1. Cut stainless-steel wire into 3-inch lengths (if available use V-portion of wire snippers to reduce rough edges at tip).

2. Soak in acetone for 10 or more minutes--then wipe off.

3. Soak in alcohol (Ethyl) for 10 or more minutes--wipe off.

4. Premake membrane solution: 7 percent cellulose nitrate by weight into amyl acetate (assume that amyl acetate density is 1 gm/cc).

5. Dip cleaned wires into membrane solution. Pull out very slowly. A slow velocity will allow the surface tension of the solution to pull excess solution off the wire.

6. Dry wires by hanging them in an oven at 65-75°C for 15 minutes.

7. Redip the tips (forming small bead at end to assure coverage of sharp edges) and let dry 15 minutes in the oven.

8. Insert membrane coated wire into the syringe end of the hypodermic needle being careful not to bend or scrape the wire on the needle. When the wire comes to the sharpened end of the needle, stop and bend remaining wire over syringe end of the needle.

9. Insert small piece of cotton inside syringe end of needle to secure wire and allow venting.

All the remaining experiments were performed with probes made according to the assembly procedure described.

Experiments 20 through 24, 30. Using TSB and P. aeruginosa.- Experiments were conducted to determine type of electrode material most applicable in manufacturing electrode detection unit (i.e., coaxial wire and needle assembly). Optimum electrode materials were found to consist of straight 28-gauge stainless-steel wire (0.014-inch diameter) and 18-gauge 1 1/2-inch, stainless-steel disposable needles. Commercially prepared coaxial tubing was also tested and showed poor adaptability to this process. Problems were encountered with these experiments until the coated center wire was obtained in stiffened and straightened form. Soft wire or wire which had been wound on spools had a tendency to short-out against the needle.

Experiments 27, 28, 31, 32, 33: System Evaluations Using Various Media and P. aeruginosa.- These experiments were designed to evaluate the detection system using various media that are used in clinical BCB. Columbia broth with 10 percent sucrose and certoid, thioglycolate, and brain-heart infusion were evaluated. All had O(+) blood added (10 percent by volume). The Columbia and thioglycolate broths were also evaluated without the addition of blood. Results from all of these experiments showed good baseline stability with good, clear deflections in output potential indicating microbe detection. Experiments using a 10 percent

blood addition achieved baseline stability in less time than experiments studied without the blood addition.

Experiment 34: Using Four-Wire Electrodes.- This experiment was designed to show the characteristic behavior of 304 stainless-steel wire in both a coated (with 7 percent Parlodion) and non-coated condition when placed in Columbia broth that has been inoculated with P. aeruginosa. There were two coated wires and two non-coated wires, all of which were placed into the same solution. One coated wire was selected as a common reference for the remaining test electrodes When tested with the other coated wire, the results indicate that both wires experienced the same changes thus producing no net change in potential. Testing the coated reference wire with respect to the non-coated wires produced the characteristic detection deflection in potential. Both responded at the same time and with the same level of potential change.

SECTION 6

RESULTS

Linear regression analysis of standard curve results indicated that both the previous cultural history of the inoculum and the medium used to measure endpoint response affected ER detection times. Using TSB as the test medium to measure ER times, cells pregrown in either TSB or M-9 gave standard curves with similar slopes, correlation coefficients, and standard errors of the estimate (figs. 4 and 5). In contrast, using EC as the test medium, TSB pregrown cells gave a standard curve with a smaller correlation coefficient and a higher standard error of the estimate (fig. 6). When transferred into EC, both M-9 and TSB pregrown cells produced standard curves with steeper slopes and greater ER time intervals compared with cells transferred into TSB (figs. 6 and 7).

A series of experiments were conducted to evaluate E. coli viability in seawater and post-starvation behavior in various test media as a function of salinity and temperature. E. coli (urinary tract) growing exponentially in M-9 was inoculated into seawater of various salinities. The greatest rate of decline in viable counts occurred during the first 2 days, rates of which increased directly with salinity (fig. 8). Although two experiments each were conducted at 20°C and 30°C, no clear effect of temperature with respect to viability was evident. Average E. coli decreases after 2 days were 0.1, 0.6, 1.1, and 1.9 log units at 20°C and 0.3, 0.7, 1.2, and 1.7 log units at 30°C in 10, 15, 25, and 30 ppt, respectively.

When the data were analyzed with respect to ER determinations, stress as previously defined was evident as a function of salinity, temperature, and test media (figs. 9 and 10). In starvation studies conducted at 20°C, stress was more pronounced in EC than TSB and increased with increasing salinity. Generally, maximum stress developed after 2 days of starvation and, thereafter, decreased. Cells starved at 30°C showed little evidence of stress except at 30 ppt salinity when transferred to EC.

A similar study was conducted using an E. coli strain isolated from estuarine water, pregrown in M-9, and starved in 10 ppt and 25 ppt seawater at 20°C. This isolate was more refractory to starvation and salinity effects than the preceding isolate which was obtained from a human host. After 15 days in seawater, there was no decrease in viable cell count in 10 ppt seawater, whereas a 0.9 log unit reduction had occurred in 25 ppt

seawater. Stress resulting from starvation at either salinity in terms of prolonged ER time was not observed (fig. 11).

A study was conducted to determine if cell death in the test media contributed to the increases in ER seen under different conditions. The observed log number of E. coli in the test medium minus the predicted E. coli number calculated from the viable count in seawater was plotted against time of starvation. With respect to the urinary tract isolate, the greatest differences between observed and anticipated bacterial levels occurred in EC as opposed to TSB (figs. 12A and 12B) and were generally accompanied by prolonged ER values (figs. 9 and 10). In contrast, the estuarine water isolate, which showed no evidence of starvation stress also showed little if any reduction in cell number upon exposure to EC or TSB (fig. 13). Although the data suggested that delayed ER times were related to cell death upon exposure primarily to the selective medium EC at the elevated temperature, all the results could not be explained solely on this basis. For instance, cell death in EC after exposure to 30 ppt seawater at 20°C and 30°C was approximately the same after 5 and 8 days starvation whereas significantly prolonged ER times were observed only at the earlier time interval. Therefore, growth in EC and TSB was examined after various exposure periods to 25 ppt seawater at 20°C. Figure 14 illustrates that upon inoculation into EC, starved cells evidenced a decline in viable count for approximately 1 hour with the lag period extended an additional hour after 2 days but not after 8 days starvation. Similar effects were not observed in TSB nor was there an apparent effect on growth rate in either medium.

A series of experiments were initiated to evaluate the impact of prestarvation history of the inoculum on viability in seawater and on the post-starvation behavior in test media at 44.5°C. The urinary tract isolate was grown under the following set of conditions: (a) exponential phase in minimal medium (M-9), (b) stationary phase in a rich medium (TSB), and (c) exponential phase in a rich medium (TSB). Treated cultures were then inoculated into 25 ppt seawater at different temperatures. Exponential phase cells from M-9 and stationary phase cells from TSB showed similar viability profiles (fig. 15). Maximum cell death occurred during the first 3 days of starvation and ranged from 0.7 to 1.2 log units. In contrast, exponentially grown TSB cells were more sensitive to starvation with a reduction of 2.3 log units over a 3-day period at 20°C and 4.2 log units after 1 day at 35°C (fig. 15C).

Similarly, cells grown exponentially in TSB, followed by starvation in seawater, showed an aberrant post-starvation behavior in test media at 44.5°C. After starvation in 25 ppt seawater at 20°C, TSB-grown cells evidenced greater die-off when introduced into either TSB or EC at 44.5°C than was previously

observed with M-9 grown bacteria (figs. 12A and 12D). Use of PBS at room temperature as diluent for serial dilution and plating may have contributed to this apparent die-off. For the cells starved at 35°C, TSB prewarmed to 35°C was used as diluent, and this apparent cell die-off was not observed (fig. 12C). Stress as measured by an increase in observed minus predicted ER time was more obvious in the TSB pregrown culture starved at 20°C than at 35°C (figs. 16 and 17). This stress occurred earlier than in the M-9 pregrown cultures, as soon as 5 hours after exposure to seawater. The degree of sublethal stress at 35°C was probably obscured by the rapid loss of viability.

The effects of cold-temperature starvation on viability and sublethal stress were determined for an E. coli isolate obtained from human feces. It should be noted that this culture differed from the other isolates in having been maintained on artificial media approximately 6 months prior to experimentation while other isolates were used immediately after isolation with laboratory maintenance not exceeding 2 months. The fecal isolate was grown in M-9 and inoculated in 25 ppt seawater at 2°C. After a 3-week starvation period, no die-off was evident with the maximum reduction of 0.4 log units occurring by 12 weeks. However, ER times increased dramatically, especially in TSB indicating the existence of sublethal stress (fig. 18). The increase in ER represented increased lag time and/or decreased growth rate since very little cell die-off occurred when the starved cells were introduced into test media at 44.5°C (fig. 19). Growth of this same fecal isolate in EC and TSB following starvation in 25 ppt seawater at 20°C revealed a longer lag time in TSB as starvation progressed (fig. 20). In contrast, growth curves of the urinary-tract isolate following starvation revealed increased lag times in EC (fig. 14). These data illustrated that length of exposure to artificial media was an important aspect to be considered in conducting seawater survival experiments.

Standard curves were developed for use in detecting stress in field isolates (figs. 21 and 22). For these studies, one part TSB pregrown inoculum was added to one part single-strength Medium A-1 or EC at 44.5°C. The ER times in Medium A-1 were shorter than those in EC for similar inoculum levels with time differences decreasing as the inoculum size increased. Introduction of a larger volume of inoculum, resulting in dilution of selective agents as well as reduction in the medium temperature probably contributed to more consistent results and earlier ER values as compared to previous standard curves in EC using TSB grown inocula (fig. 6).

Similar results were noted when field samples were diluted 1:1 into single-strength test media. The ER times occurred 2 to 11 hours sooner in A-1 than in EC with a mean difference of approximately 5 hours. When compared to the standard curve, field samples

inoculated into A-1 gave ER values extended by mean values of 0.6 and 0.9 hour for samples collected from waters with mean temperatures of 14°C and 29°, respectively. Since the standard error of the estimated $y(S_{\hat{y}})$ at t_{005} for the A-1 standard curve was ±0.7 hour these results did not indicate that the cells had been greatly stressed at either temperature range.

Corresponding field samples processed in EC produced ER times with mean deviations from the predicted times of +1.65 hours for samples taken from cooler waters ($\bar{X}$ = 14°C) and -1.78 hours for samples taken from warmer ($\bar{X}$ = 29°C) waters. Since the standard error of the estimated y for the standard curve in EC was ±1.14, and since the number of sampling points was limited, the question as to whether or not fecal coliforms in our environmental samples were stressed remains unresolved.

DISCUSSION

Fecal coliforms introduced into the estuarine environment are exposed to various physiochemical and biological factors which contribute to viability losses. For example, dilution, adsorption to particulates (ref. 12), sedimentation, sunlight (refs. 6 and 13), organics (refs. 14, 24, 26, 30, 35), salinity (ref. 9), temperature (refs. 12, 25, 36), dissolved oxygen (ref. 12), heavy metal toxicity (ref. 17), algae (ref. 21), bacteria (refs. 18, 22, 23, 29), bacteriophage (ref. 10), and protozoa (refs. 11, 22), have all been discussed. In addition, researchers have suggested that a portion of the coliform population may be sublethally stressed following exposure to the aquatic environment (refs. 7, 39).

Studies at the Virginia Institute of Marine Science have been limited to the effects of three variables--salinity, temperature, and previous cultural history on viability and stress of fecal coliforms. Although the data showed that viability of *E. coli* was inversely related to salinity, a similar relationship with respect to temperature was not as evident. Cells at 15, 20, 30, and 35°C showed similar viability profiles, although at 35°C, there was a continued loss of viability after the initial sharp decline in bacterial numbers. A negligible loss of viability was demonstrated after 85 days of exposure to 2°C seawater (25 ppt). Cryptic growth may have occurred thus obscuring viability losses since the initial bacterial levels in seawater in all of these experiments ranged from approximately 10^6 to 5×10^7 cells/ml.

The literature suggests that *Enterobacter* (as *Aerobacter*) *aerogens* exposed to either distilled water or buffer solutions at low temperatures displays loss of permeability control which results in leakage of cellular constituents with a progressive

and rapid loss of viability (refs. 33 and 34). In the absence of pronounced cell death in our experiment at 2°C, it could be hypothesized that the hyperosmotic environment provided by 25 ppt seawater protected chilled cells by decreasing the diffusion rate of cellular constituents.

The most dramatic differences between viability profiles were observed to be a function of cultural history. Cells harvested during exponential phase from a rich medium (TSB) were more sensitive to seawater than either exponential cells from minimal media M-9, or stationary phase cells from TSB. Although it is tenuous to extrapolate laboratory results to the estuarine environment, our findings are of value to those concerned with estuarine modeling studies involving the determination of coliform die-off coefficients. The previous history of coliforms introduced to the estuary could affect survival. For example, the physiological fate of E. coli derived from untreated sewage or other rich organic millieux, as opposed to immediate storm water runoff, may differ when discharged into the estuarine environment.

Little actually is known about the physiological changes that occur in E. coli in the estuarine environment. There is no doubt that the organisms develop different levels of sublethal stress depending upon the conditions encountered. Reports in the literature suggest that difficulties in analyzing water samples using membrane filter techniques may be due to the fragility of sublethally stressed organisms. These organisms may undergo mechanical destruction during the filtration process or be killed by selective media used in the enumeration procedure (ref. 15). New techniques have been devised to deal with sublethally stressed organisms--some involved a period of resuscitation in a nonselective media (refs. 7 and 37); others use a sandwich technique involving nonselective and selective media (refs. 28 and 32).

The electrochemical detection method (ECDM) assays the time required for the inoculum to become metabolically active and reach a critical cell density. Wilkins and Boykin (ref. 3) suggested that ECDM might provide a rapid and inexpensive method for monitoring coliform levels in the estuarine environment. However, it was not clear how the presence of stressed cells would affect the reliability of the ECDM results. The VIMS studies were concerned with what conditions of salinity, temperature, or previous cultural history would produce the highest levels of sublethal stress as determined by ECDM. In addition, estuarine water samples were collected for analysis by ECDM and by standard MPN techniques to determine if stress in environmental coliforms could be detected.

It was obvious from the data collected that ECDM could detect stress induced in laboratory coliform populations. Stress was particularly evident in cells exposed to 25 and 30 ppt seawater at 20°C. At 30°C, stress was detected only in those cells exposed to the highest salinity (30 ppt). When kept in seawater at 2°C (25 ppt), cells developed significant stress only after a long exposure even though there was little loss in viability. Pronounced stress was also observed in samples of E. coli pregrown in a rich medium. The data suggested that prolonged ER times may be due to immediate cell die-off as the starved cells encounter the test media at 44.5°C and/or an increase in lag time.

When a limited number of environmental samples were analyzed by the ECDM method, stress was not apparent. All of the VIMS samples were collected in the immediate vicinity of a known point pollution source. The organisms sampled were likely to have had a short residence time in the estuarine environment and this could explain the absence of pronounced stress.

REFERENCES

1. American Public Health Association, Standard Methods for the Examination of Water and Wastewater. Fourteenth Edition, 1975.

2. Wilkins, Judd R., Glenn E. Stoner, and Elizabeth H. Boykin. Microbial Detection Method Based on Sensing Molecular Hydrogen. Applied Microbiology. vol. 27, no. 5, May 1974, pp. 949-952.

3. Wilkins, Judd R., and Elizabeth H. Boykin. Electrochemical Method for Early Detection and Monitoring of Coliforms. J. American Water Works Assoc., vol. 68, no. 5, May 1976, pp. 257-263.

4. Grana, David C., and Judd R. Wilkins. Description and Field Test Results of an In situ Coliform Monitoring System. NASA TP-1334, January 1979.

5. Andrews, W. H., and M. W. Presnell. Rapid Recovery of Escherichia coli from Estuarine Water. Appl. Microbiol. 23:521-523, 1972.

6. Anson, A. E., and G. C. Ware. Laboratory Studies on the Effect of the Container on the Mortality of E. coli in Sea Water. Water Res. 9: 895-899, 1975.

7. Bissonnette, G. K., J. J. Jezeski, G. A. McFeters, and D. G. Stuart. Influence of Environmental Stress on Enumeration of Indicator Bacteria from Natural Water. Appl. Microbiol. 29: 186-194, 1975.

8. Braswell, J. R., and A. W. Hoadley. Recovery of Escherichia coli from Chlorinated Secondary Sewage. Appl. Microbiol. 28:328-329, 1974.

9. Carlucci, A. F., and D. Pramer. An Evaluation of Factors Affecting the Survival of Escherichia coli in Sea Water. II. Salinity, pH, and Nutrients. Appl. Microbiol. 8:247-250, 1960a.

10. Carlucci, A. F., and D. Pramer. An Evaluation of Factors Affecting the Survival of Escherichia coli in Sea Water. IV. Bacteriophages. Appl. Microbiol. 8:254-256, 1960b.

11. Enzinger, R. M., and R. C. Cooper. Role of Bacteria and Protozoa in the Removal of Escherichia coli from Estuarine Waters. Appl. Environ. Microbiol. 31:758-763, 1976.

12. Faust, Maria A., A. E. Aotaky, and M. L. Hargadon. Effect of Physical Parameters on the In situ Survival of Escherichia coli MC-6 in an Estuarine Environment. Appl. Microbiol. 30:800-806, 1975.

13. Gameson, A. L. H., and J. R. Saxon. Field Studies on Effect of Daylight on Mortality of Coliform Bacteria. Water Res. 1: 279-295, 1967.

14. Gerba, C. P., and J. S. McLeod. Effect of Sediments on the Survival of Escherichia coli in Marine Waters. Appl. Environ. Microbiol. 32:114-120, 1976.

15. Hoadley, A. W. Effect of Injury on the Recovery of Indicator Bacteria on Membrane Filters. In Symposium on the Recovery of Indicator Organisms Employing Membrane Filters, R. Bordner, C. Frith, and J. Winter, eds., Environmental Protection Agency, Cincinnati, Ohio. 1977, pp. 34-40.

16. Hoadley. A. W., and C. M. Cheng. The Recovery of Indicator Bacteria on Selective Media. J. Appl. Bacteriol. 37:45-57, 1974.

17. Jones, G. E. Effect of Chelating Agents on the Growth of Escherichia coli in Sea Water. J. Bacteriol. 3: 483-499, 1964.

18. Ketchum, B. H., C. L. Carey, and M. Briggs. Preliminary Studies on the Viability and Dispersal of Coliform Bacteria in the Sea. In Limnological Aspects of Water Supply and Waste Disposal, Publ. Amer. Assoc. Adv. Sci., 1949, pp. 64-73.

19. Klein, D. A. and S. Wu. Stress: A Factor to be Considered in Heterotrophic Microorganism Enumeration from Aquatic Environments. Appl. Microbiol. 27:429-431, 1974.

20. Milbauer, R., and N. Grossowicz. Reactivation of Chlorine-Inactivated Escherichia coli. Appl.Microbiol. 7:67-70, 1959.

21. Mitchell, R. Factors Affecting the Decline of Non-Marine Microorganisms in Sea Water. Water Res. 2:535-543, 1968.

22. Mitchell, R. Destruction of Bacteria and Viruses in Sea Water. J. Sanitary Engineering Division. Proceedings of the Am. Soc. Civil Engineers. 1971, pp. 425-432.

23. Mitchell, R., and J. C. Morris. The Fate of Intestinal Bacteria in the Sea. In S. H. Jenkins (ed.), Advances in Water Pollution Research. Pergamon Press, London, 1969, pp. 811-821.

24. Moebus, K. Factors Affecting Survival of Test Bacteria in Sea Water: Marine Bacteria, Test Bacteria and Solid Surfaces. Helgolander wiss. Meeresunters. 23:271-285, 1972.

25. Nusbaum, I., and R. M. Garver. Survival of Coliform Organisms in Pacific Ocean Coastal Waters. Sewage Ind. Wastes 27: 1383-1390, 1955.

26. Orlob, G. T. Viability of Sewage Bacteria in Sea Water. Sewage Ind. Wastes 28: 1147-1167, 1956.

27. Postgate, J. R., and J. R. Hunter. The Survival of Starved Bacteria. J. Gen. Microbiol. 29:233-263, 1962.

28. Rose, R. E., E. E. Geldreich, and W. Litsky. Improved Membrane Filter Method for Fecal Coliform Analysis. Appl. Microbiol. 29:532-536, 1975.

29. Rosenfeld, W. D., and C. E. ZoBell. Antibiotic Production by Marine Organisms. J. Bacteriol. 54:393-398, 1947.

30. Savage, H. P., and N. B. Hanes. Toxicity of Sea Water to Coliform Bacteria. J. Water Pollut. Control. 43:854-861, 1971.

31. Simpson, G., A. Roe, and R. C. Lewontin. Quantitative Zoology. Chapter 11, Correlation and Regression. Harcourt, Brace, and World, Inc., New York, New York. 1960.

32. Speck, M. L., B. Ray, and R. B. Read, Jr. Repair and Enumeration of Injured Coliforms by a Plating Procedure. Appl. Microbiol. 29: 549-550, 1975.

33. Strange, R. E., and F. A. Dark. Effect of Chilling on Aerobacter aerogenes in Aqueous Suspension. J. Gen. Microbiol. 29: 719-370, 1962.

34. Tempest, D. W., and R. E. Strange. Variation in Content and Distribution of Magnesium, and its Influence on Survival in Aerobacter aerogenes Grown in a Chemostat. J. Gen. Microbiol. 44: 273-279, 1966.

35. Vaccaro, R. F., M. P. Briggs, C. L. Carey, and B. W. Ketchum. Viability of Escherichia coli in Sea Water. Am. J. Publ. Hlth, 40: 1257-1266, 1950.

36. Vasconcelos, G. J., and R. G. Swartz. Survival of Bacteria in Sea Water using a Diffusion Chamber Apparatus In situ. Appl. Environ. Microbiol. 31:913-920, 1976.

37. Warseck, M., B. Ray, and M. L. Speck. Repair and Enumeration of Injured Coliforms in Frozen Foods. Appl. Microbiol. 26:919-924, 1973.

38. Wilkins, J. R. Use of Platinum Electrodes for the Electrochemical Detection of Bacteria. Appl. and Environ. Micro. 36: 683-689, 1978.

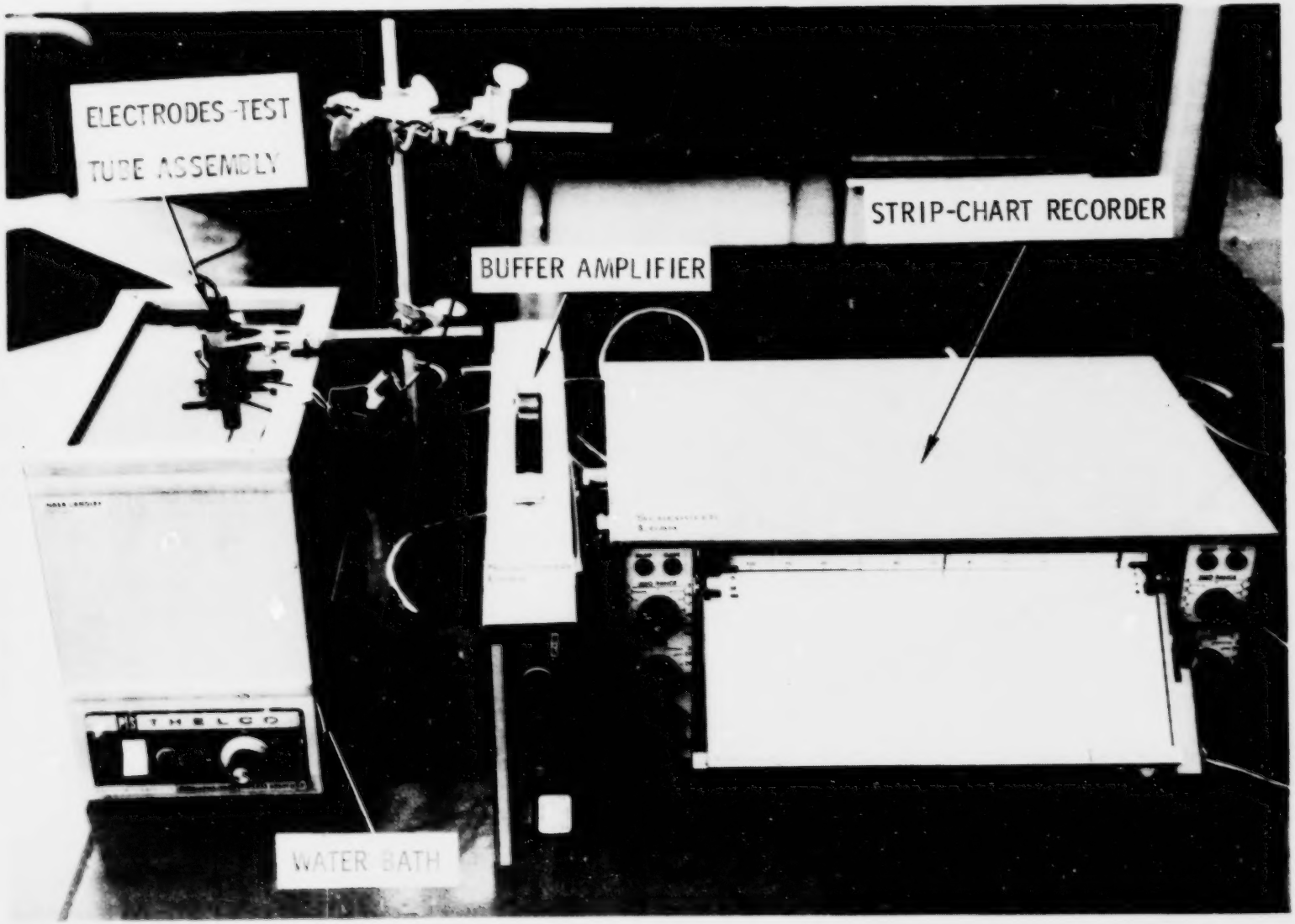

FIGURE 1. - EXPERIMENTAL SETUP FOR PERFORMING HYDROGEN RESPONSE MEASUREMENTS.

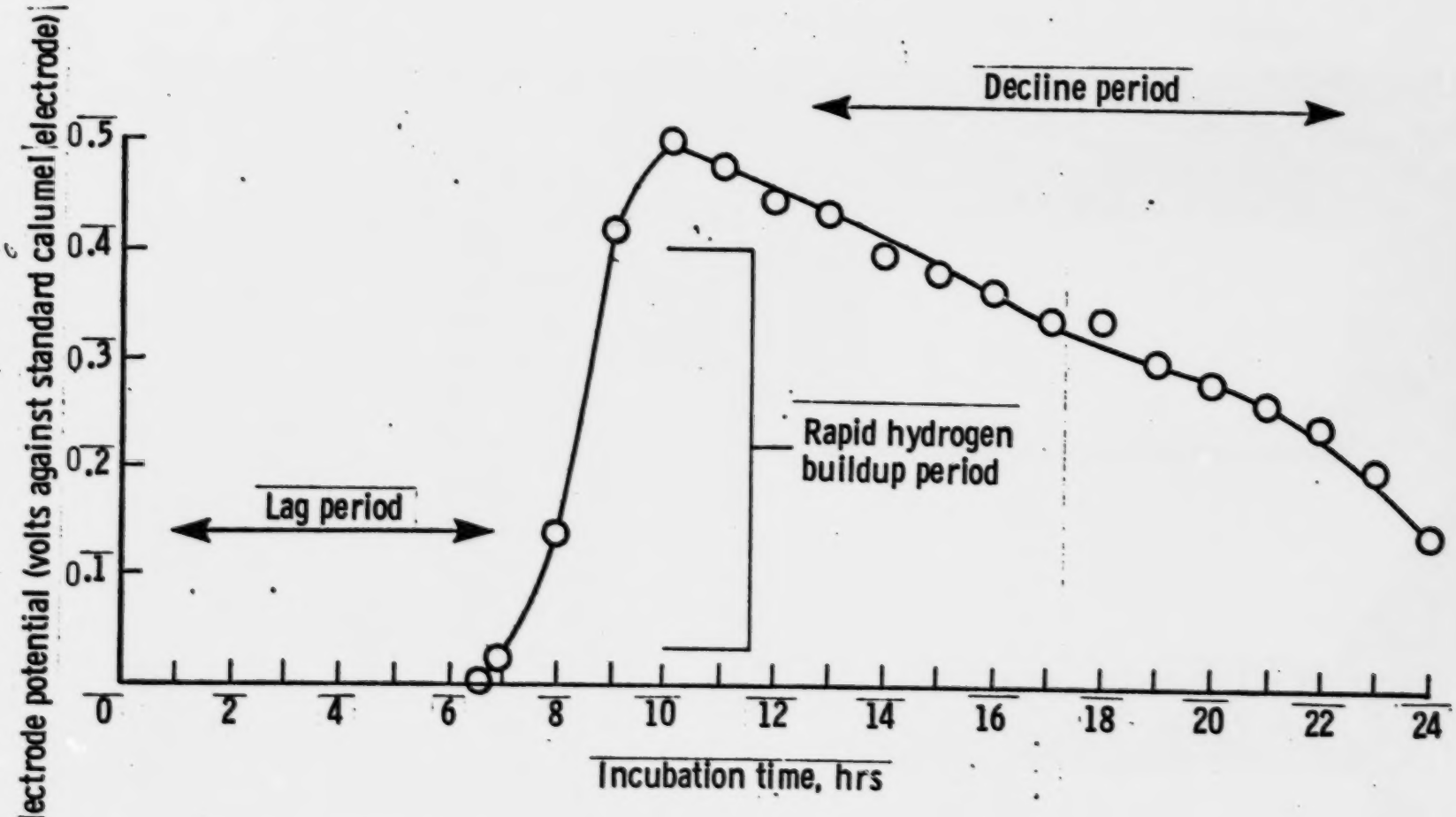

Figure 2. - Strip-chart tracing of hydrogen response curve for 1.9×10^0 cells/ 100 ml

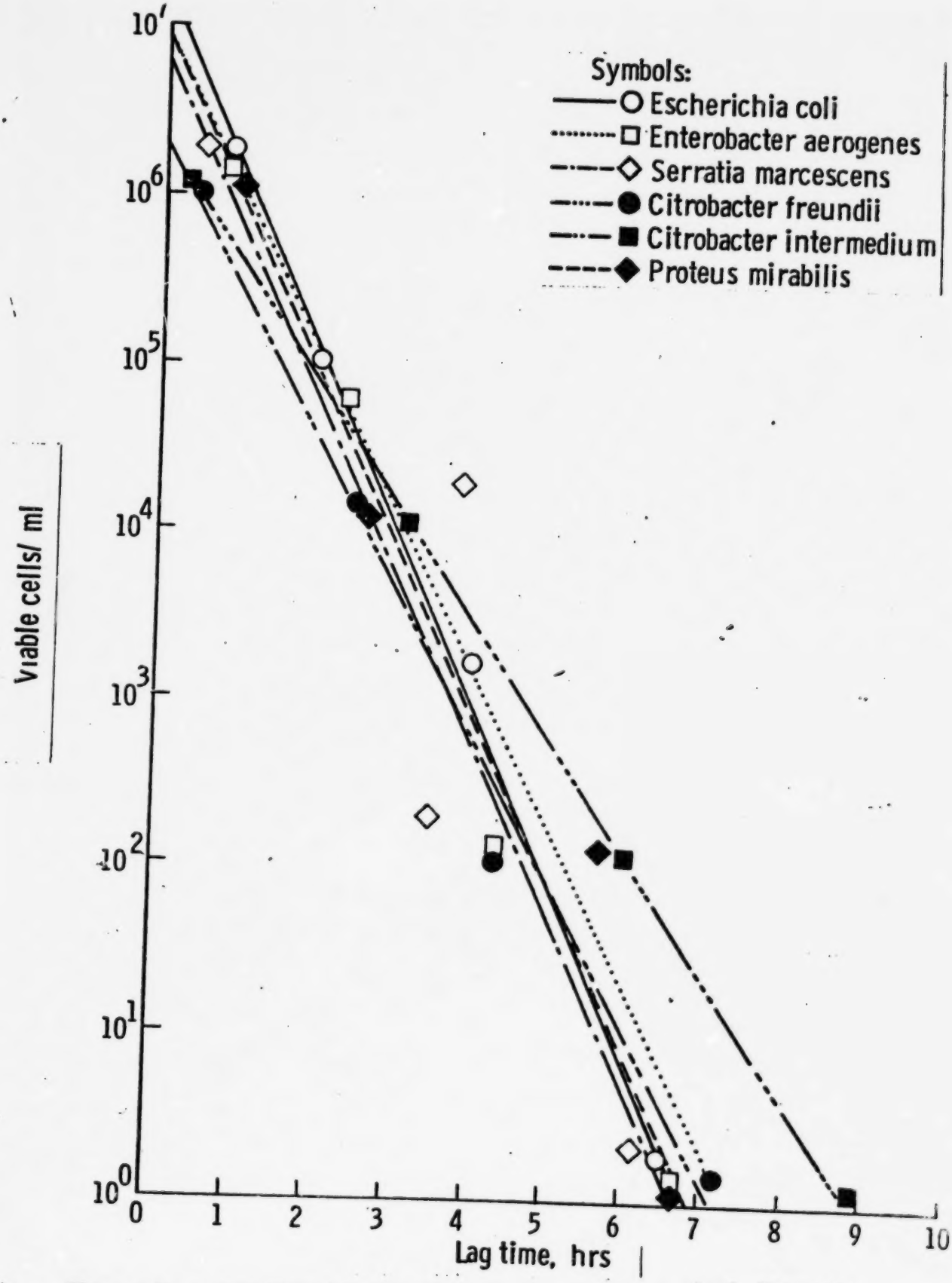

Figure 3. - Relationship between inoculum size and length of lag period. (Correlation coefficients for the lines fitted by the method of least squares.)

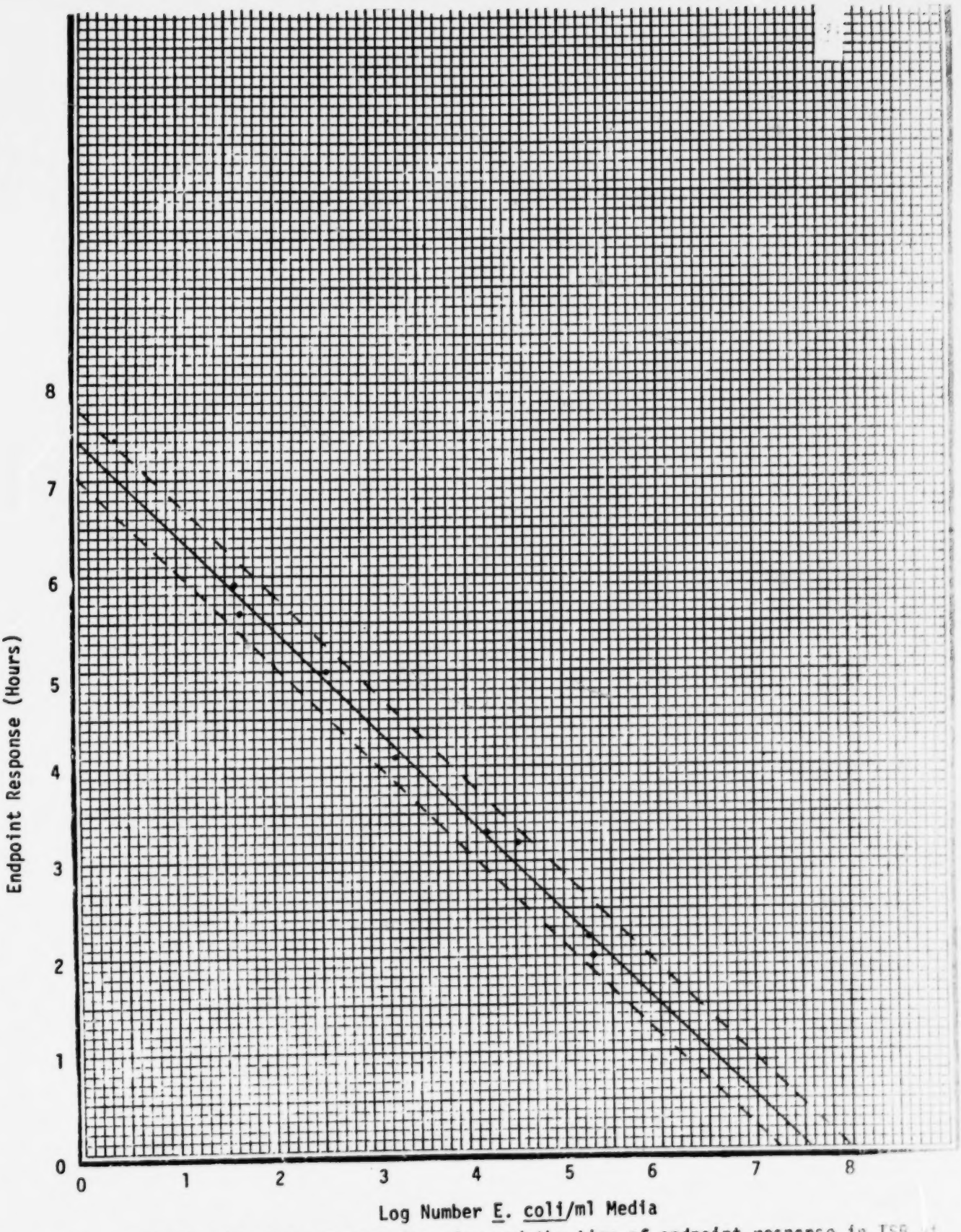

Figure 4. Relationship between inoculum size and the time of endpoint response in TSB at 44.5°C when inocula (urinary tract isolate) were pregrown in TSB at 35°C. A linear least-squares regression calculation gave a correlation coefficient of -0.99 (N = 8) with an intercept of 7.39 and a slope of -0.98. The standard error of estimate at the 95 percent confidence level was ± 0.37.

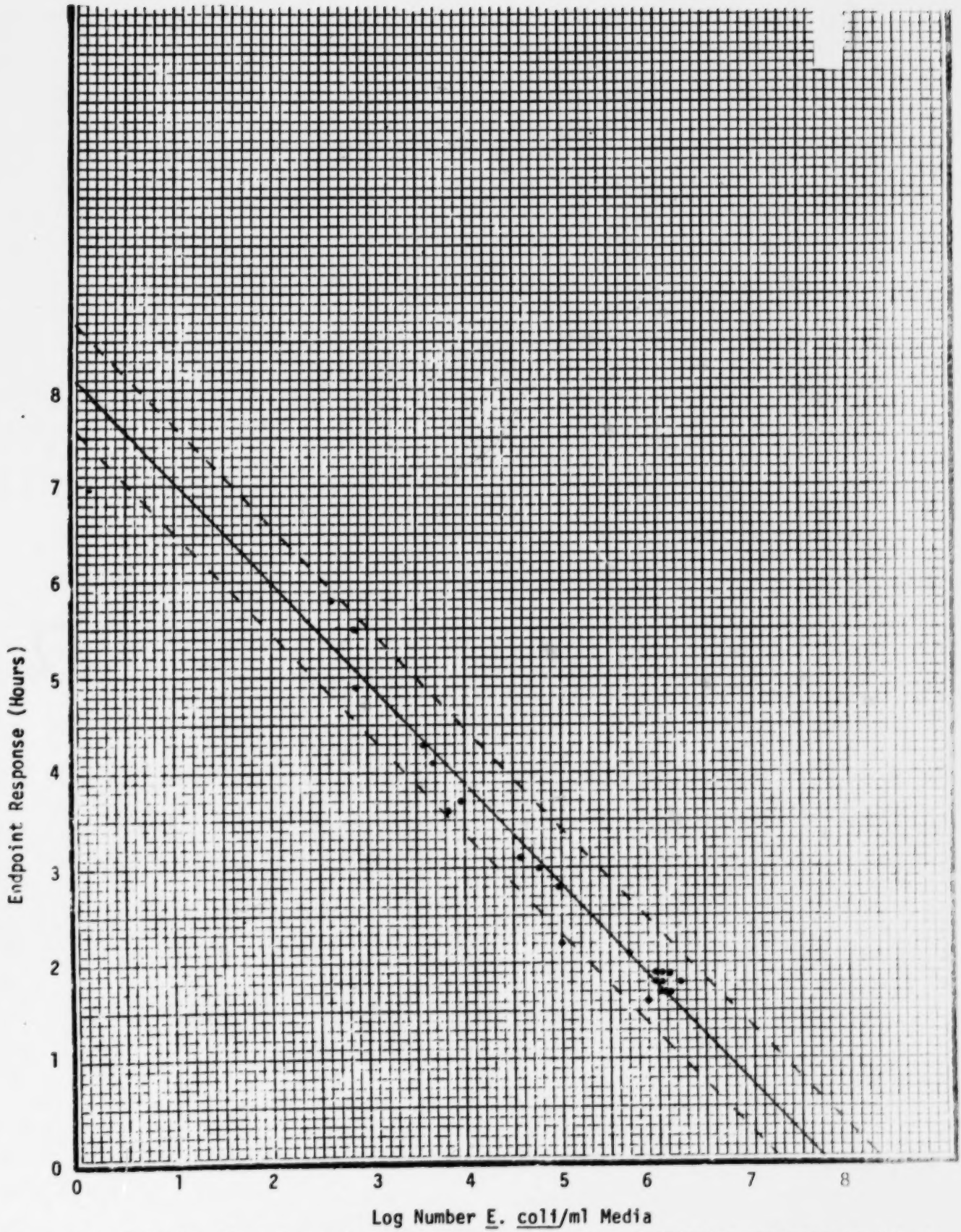

Figure 5: Relationship between inoculum size and the time of endpoint response in TSB at 44.5°C when inocula (urinary tract isolate) were pregrown in M-9 medium at 35°C. A linear least-squares regression calculation gave a correlation coefficient of -0.98 (N = 22) with an intercept of 8.07 and a slope of -1.05. The standard error of estimate at the 95 percent confidence level was ± 0.54.

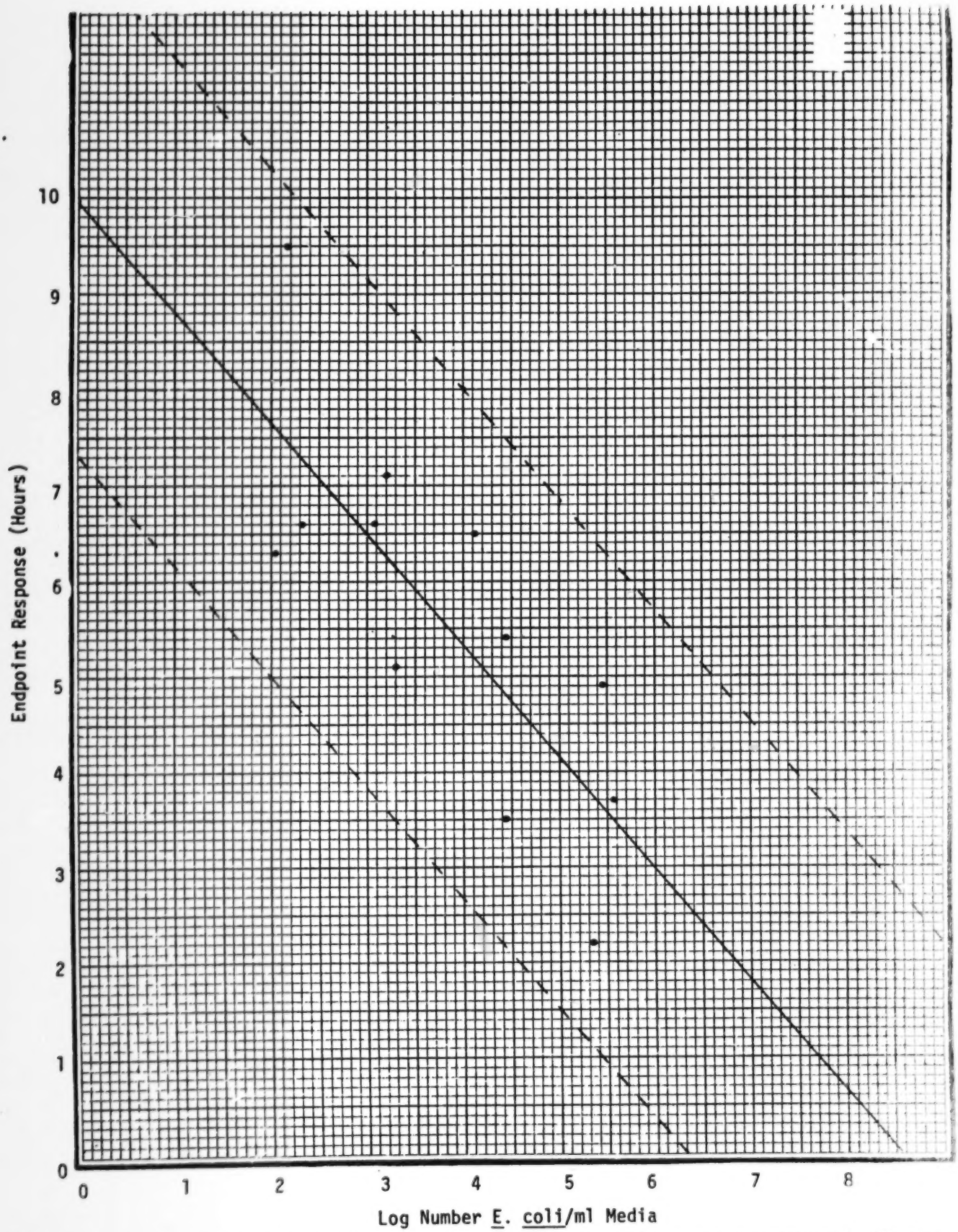

Figure 6. Relationship between inoculum size and the time of endpoint response in EC at 44.5°C when inocula (urinary tract isolate) were pregrown in TSB at 35°C. A linear least-squares regression calculation gave a correlation coefficient of -0.77 (N = 12) with an intercept of 9.97 and a slope of -1.16. The standard error of estimate at the 95 percent confidence level was + 2.67.

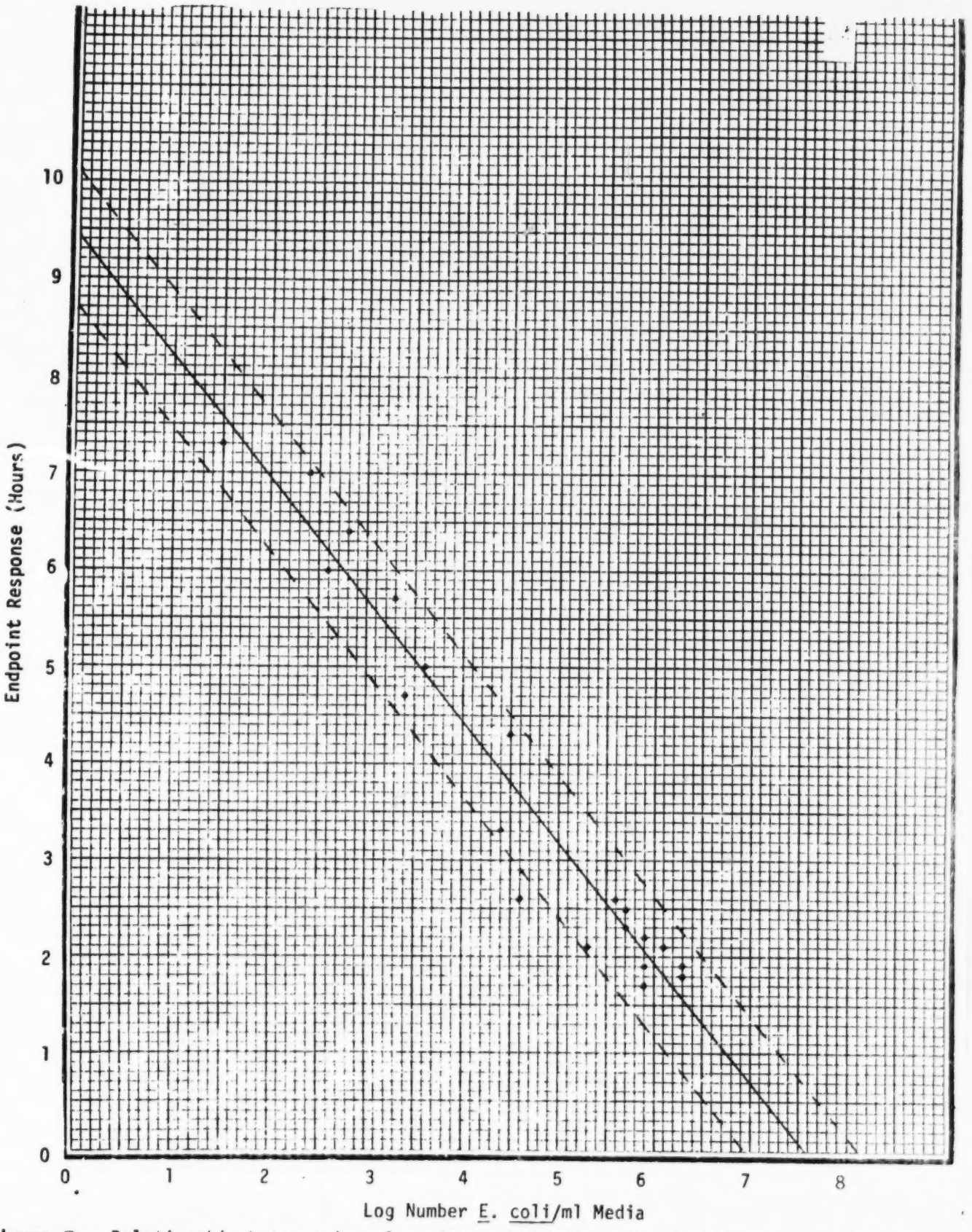

Figure 7. Relationship between inoculum size and the time of endpoint response in EC at 44.5°C when inocula (urinary tract isolate) were pregrown in M-9 medium at 35°C. A linear least-squares regression calculation gave a correlation coefficent of -0.97 (N = 20) with an intercept of 9.41 and a slope of -1.25. The standard error of estimate at the 95 percent confidence level was ± 0.73.

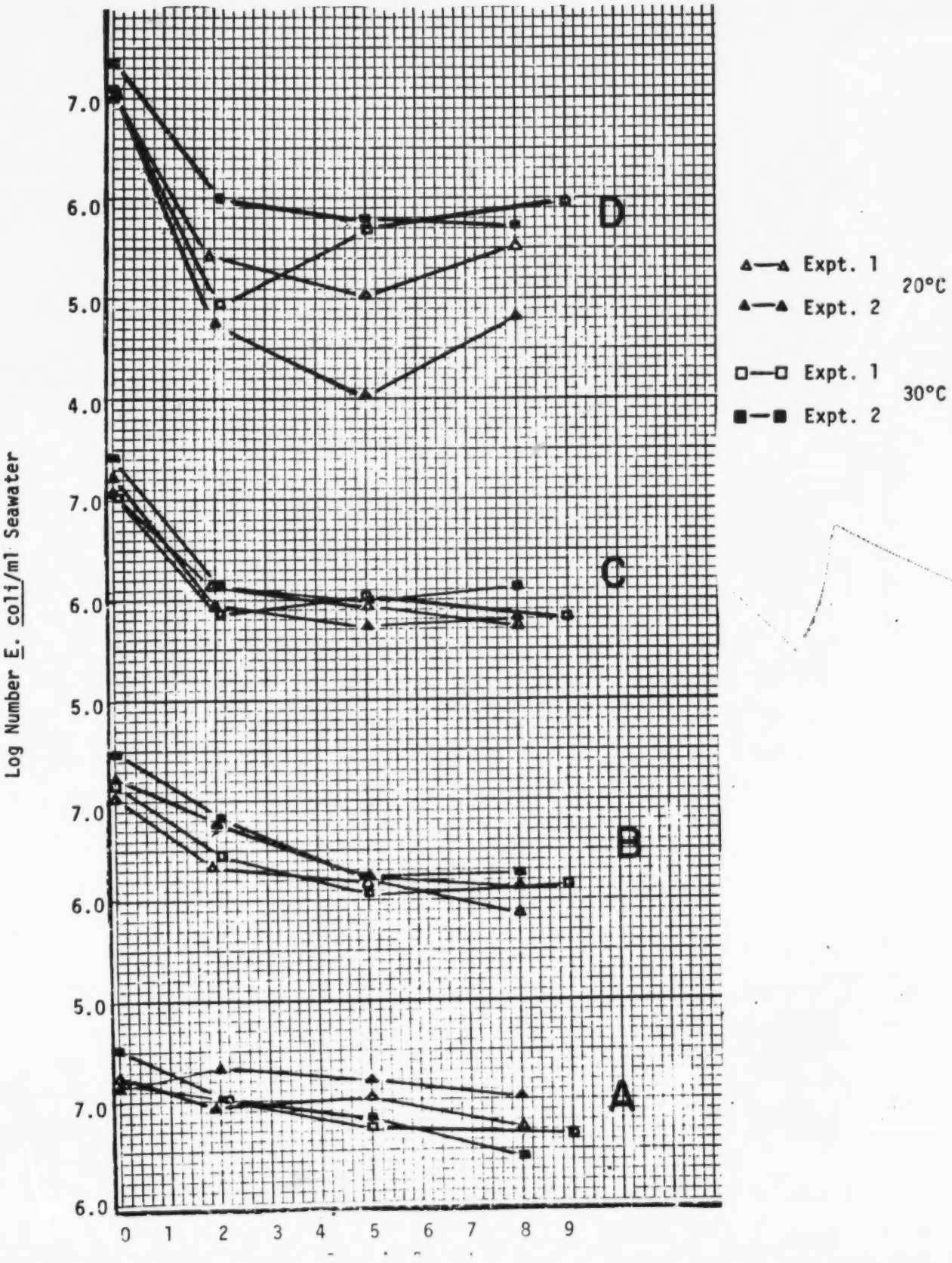

Figure 8. Comparison of viability of E. coli at 20°C and 30°C at various salinities. Prior to starvation the isolate (urinary tract) was pregrown in M-9 medium at 35°C. Salinity: A=10 ppt, B=15 ppt; C= 25 ppt, and D = 30 ppt.

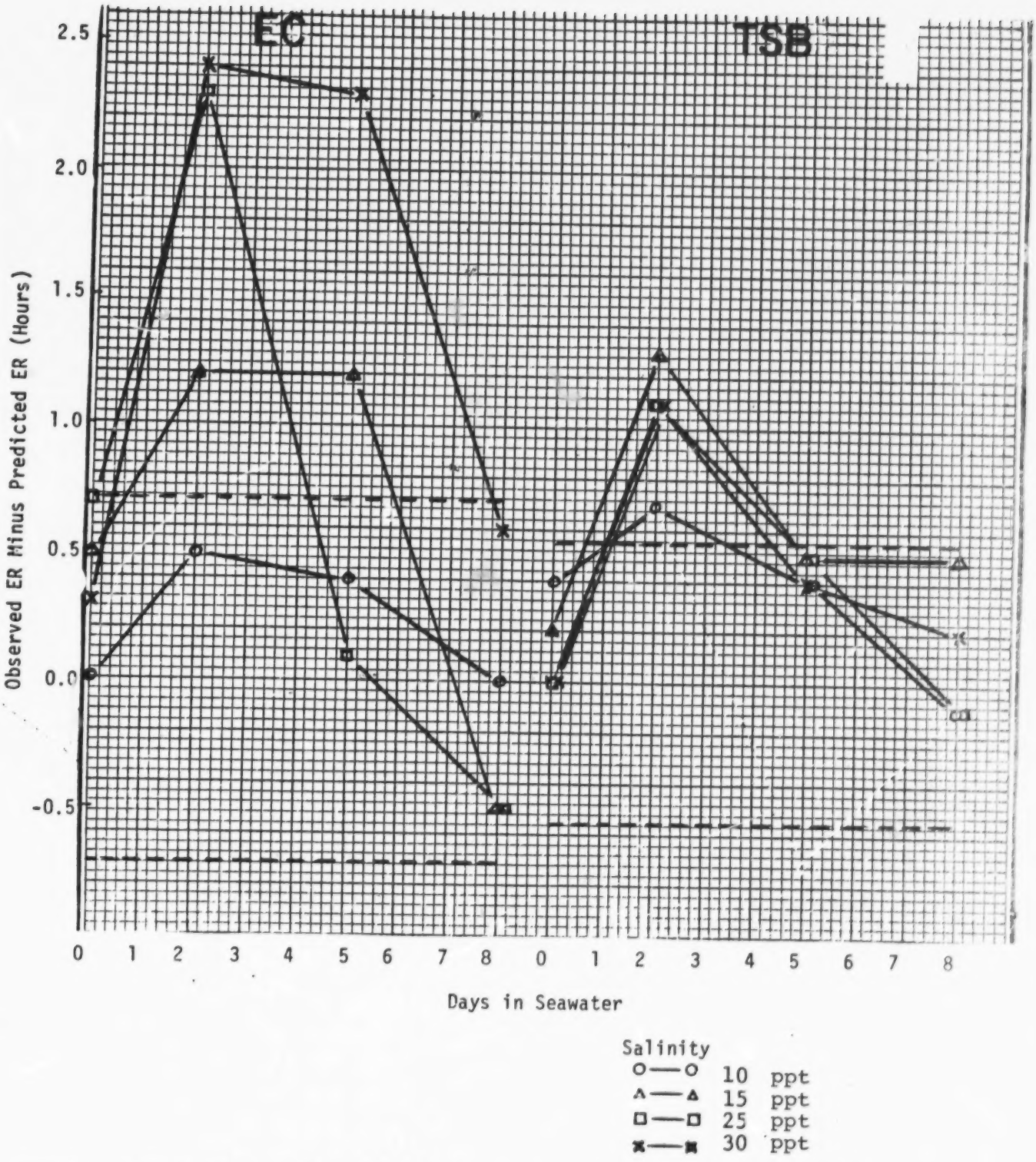

Figure 9. Effect of starvation at 20°C in seawater of various salinities on the endpoint response (ER) of E. coli inoculated into either EC or TSB at 44.5°C. Prior to starvation the isolate (urinary tract) was pregrown in M-9 medium at 35°C. Viable counts determined using PBS diluent at room temperature.

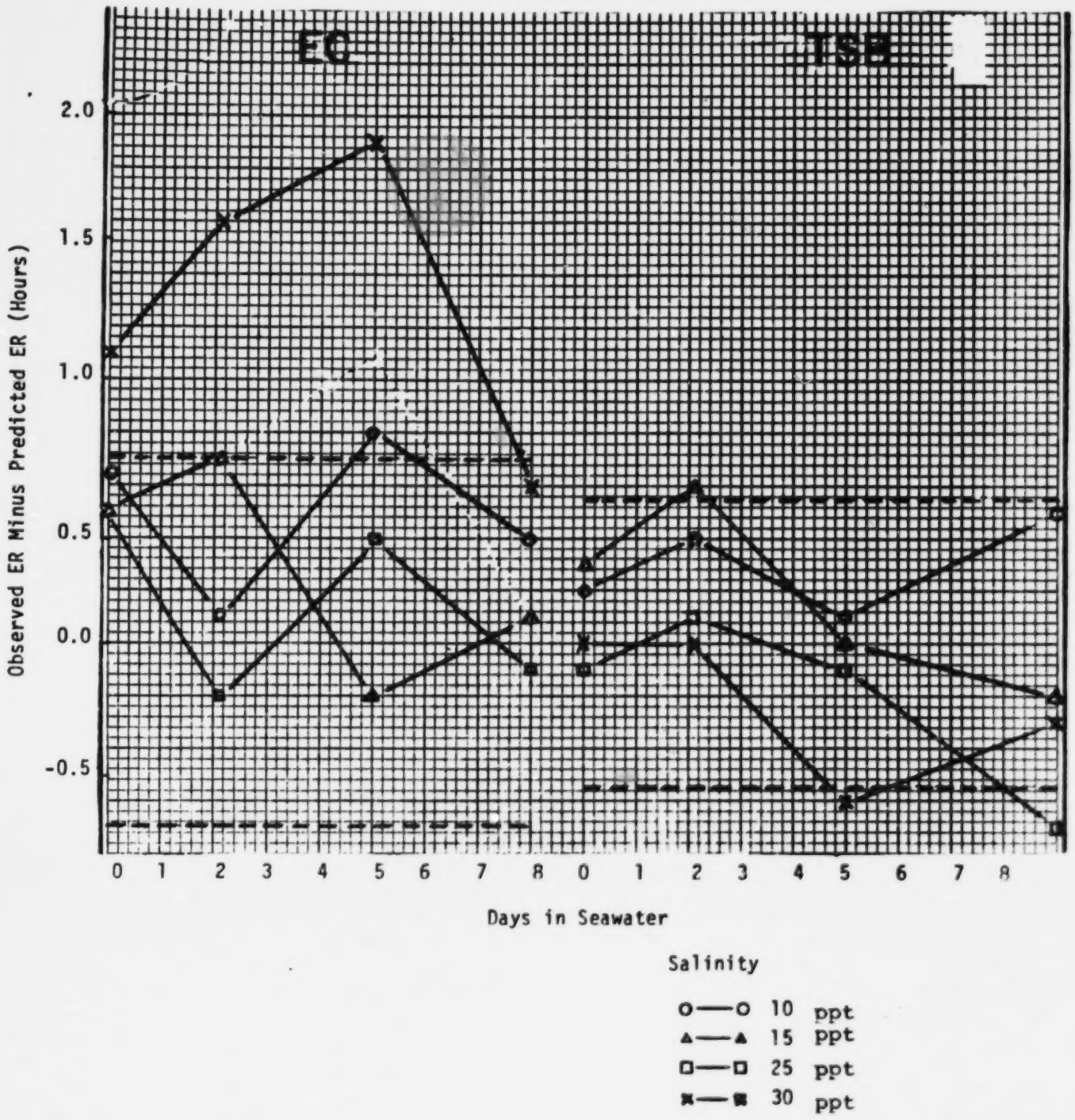

Figure 10. Effect of starvation at 30°C in seawater of various salinities on the endpoint response (ER) of E. coli inoculated into either EC or TSB at 44.5°C. Prior to starvation the isolate (urinary tract) was pregrown in M-9 medium at 35°C. Viable counts determined using PBS diluent at room temperature.

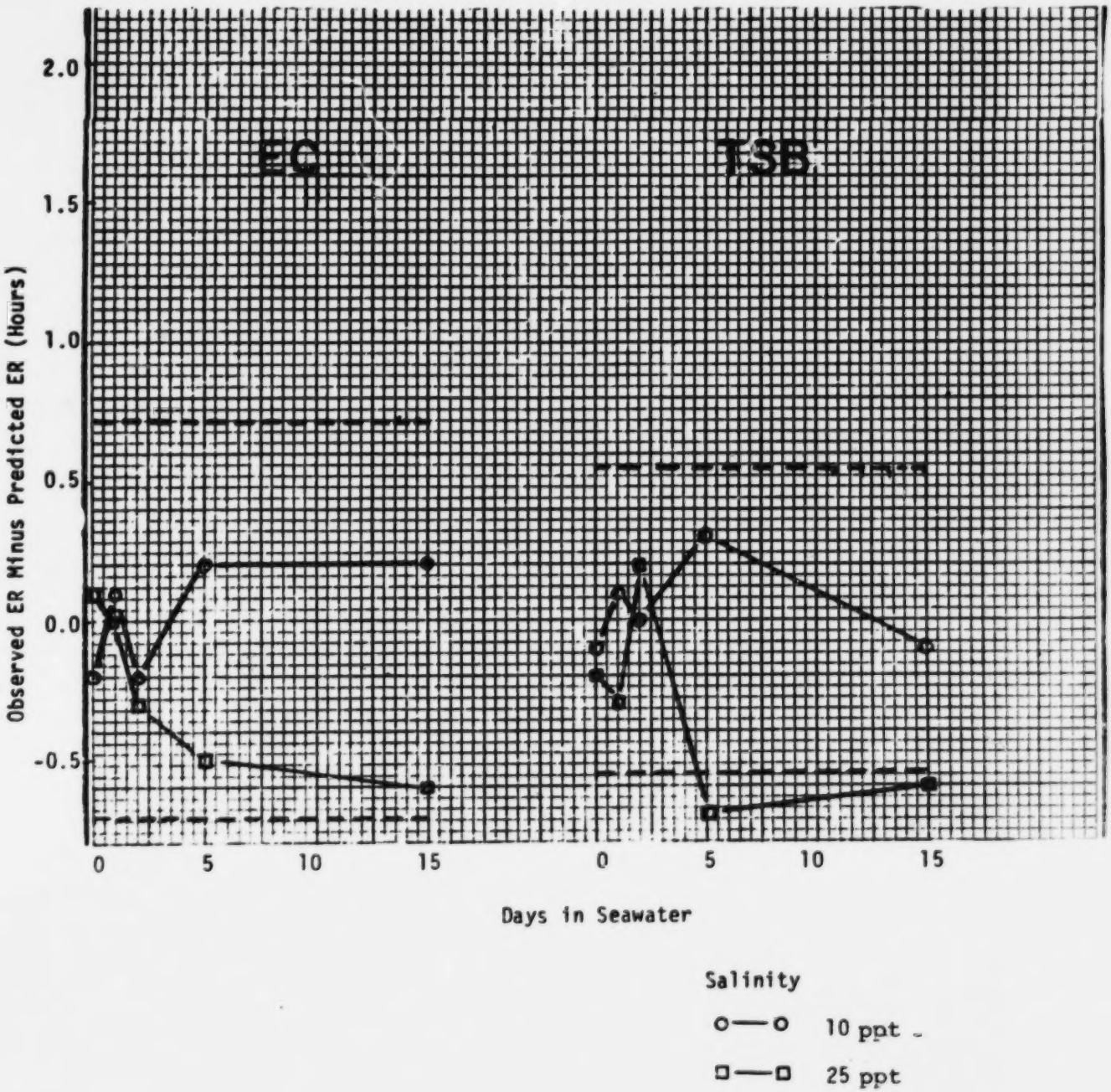

Figure 11. Effect of starvation at 20°C in seawater (10 ppt and 25 ppt) on the endpoint response (ER) of E. coli inoculated into either EC or TSB at 44.5°C. Prior to starvation the isolate (estuarine water) was pregrown in M-9 medium at 35°C. Viable counts determined using PBS diluent at room temperature.

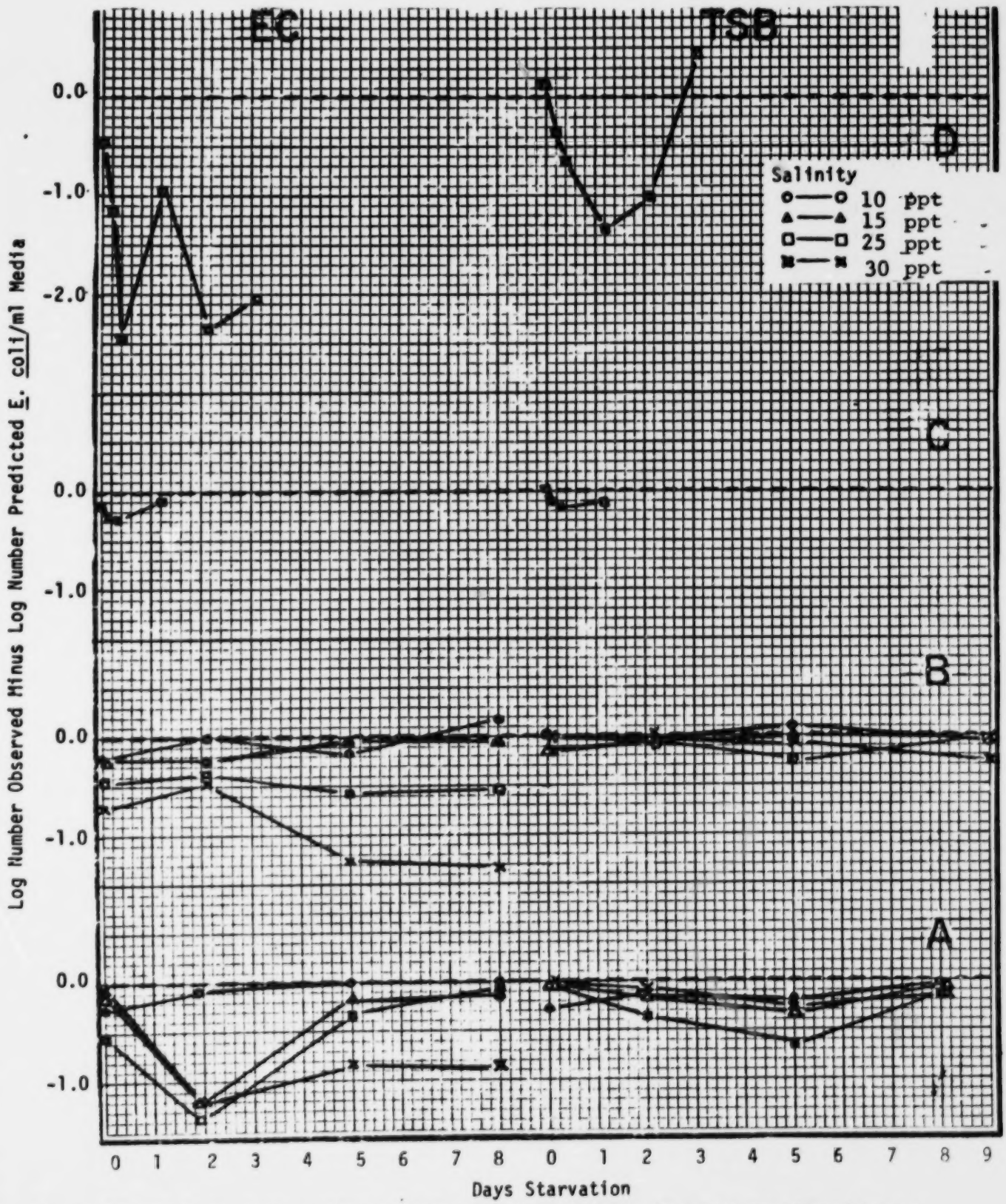

Figure 12. Effect of starvation at different temperatures in seawater of various salinities on the enumeration of E. coli (urinary tract isolate) immediately following a ten-fold dilution into either EC or TSB at 44.5°C. Bacteria pregrown in M-9: A. Seawater temperature = 20°C, PBS diluent at room temperature and B. Seawater temperature = 30°C, PBS diluent at room temperature. Bacteria pregrown in TSB: C. Seawater temperature = 35°C, TSB diluent at 35°C and D. Seawater temperature = 20°C, PBS diluent at room temperature.

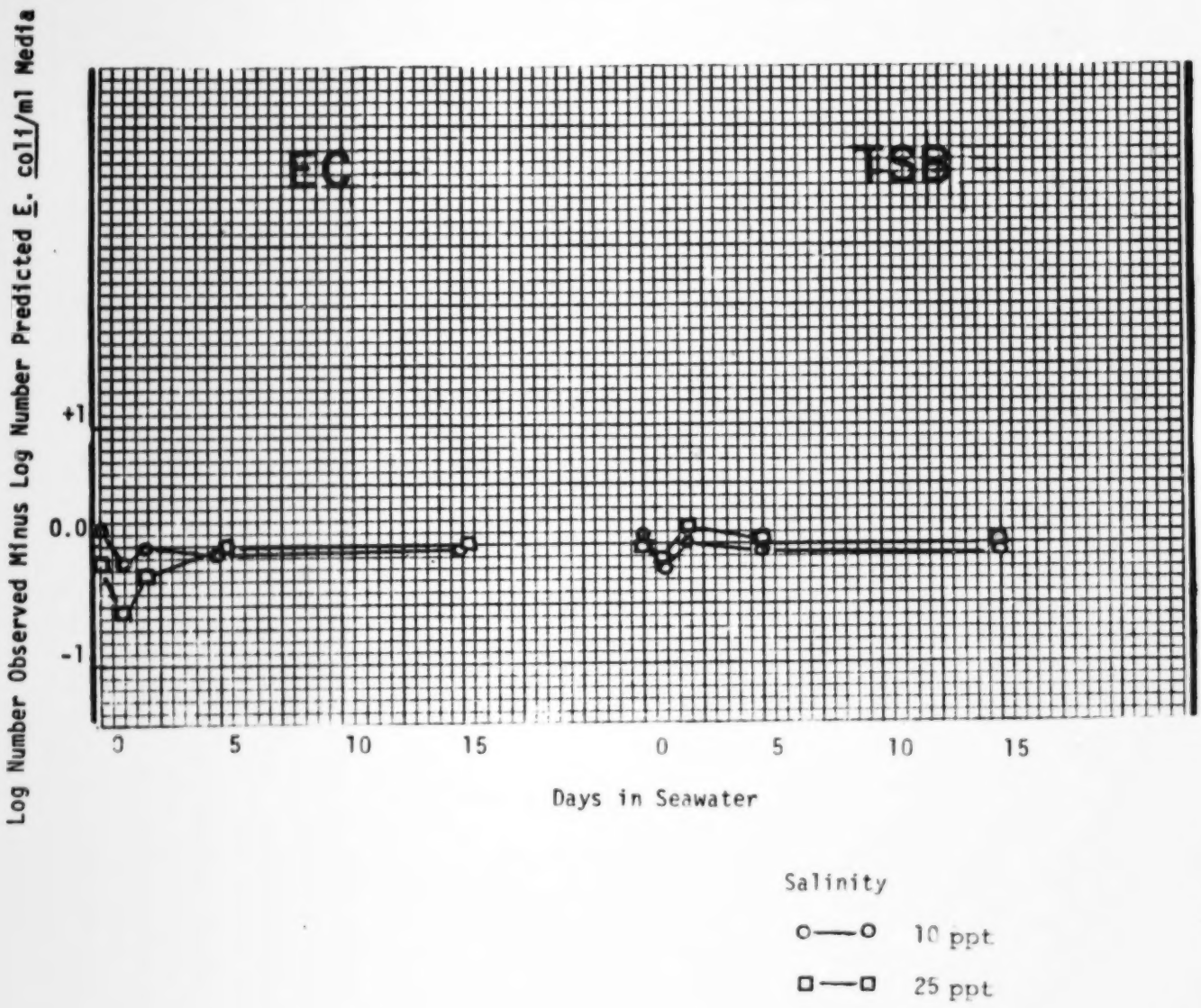

Figure 13. Effect of starvation at 20°C in seawater (10 ppt and 25 ppt) on the enumeration of E. coli immediately following dilution into either EC or TSB at 44.5°C. Prior to starvation the isolate (estuarine water) was pregrown in M-9 medium at 35°C. Viable counts determined using PBS diluent at room temperature.

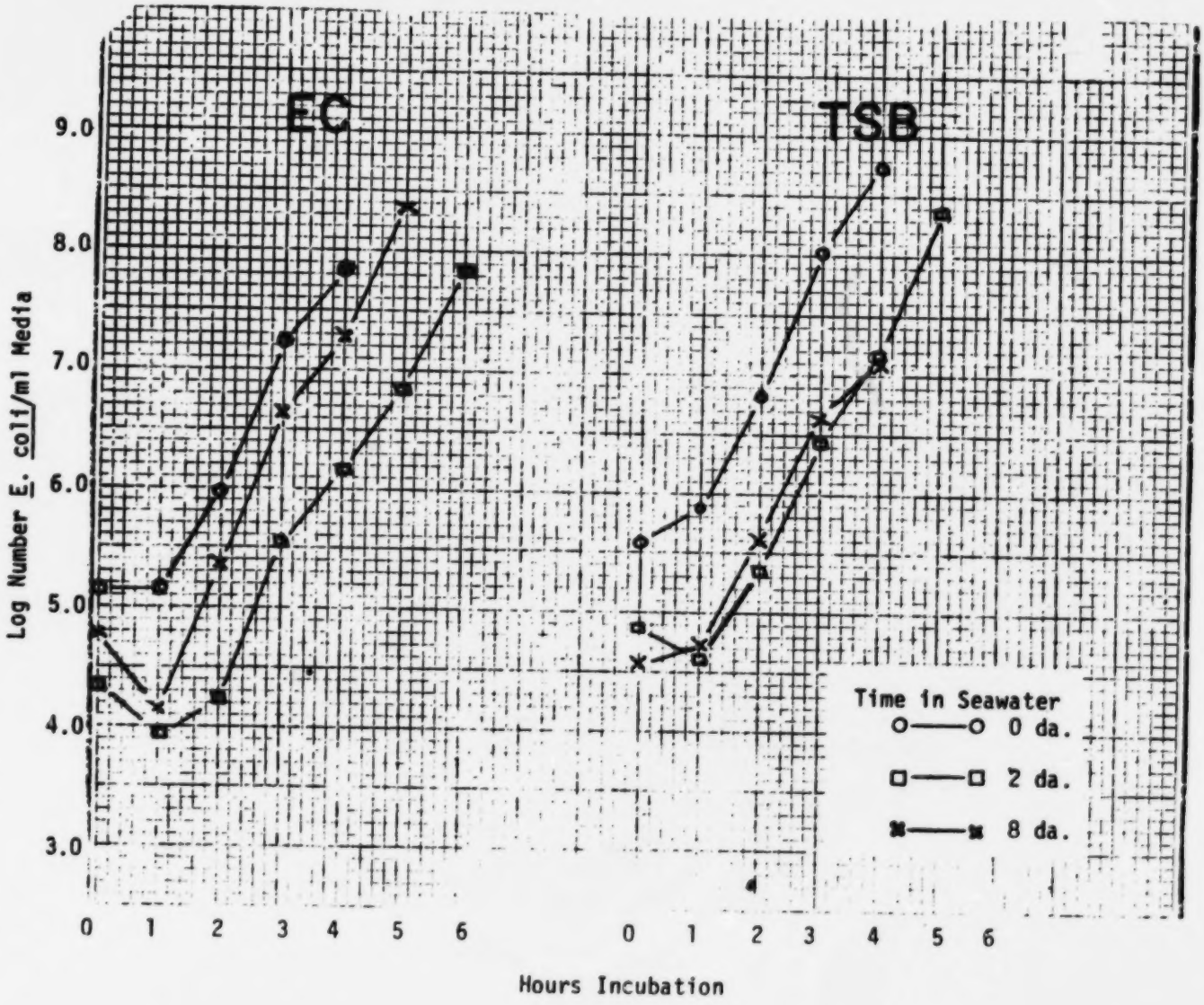

Figure 14. Growth of E. coli in EC and TSB, 44.5°C after 0, 2, and 8 days starvation at 20°C in seawater (25 ppt). Prior to starvation the isolate (urinary tract) was pregrown in M-9 medium at 35°C.

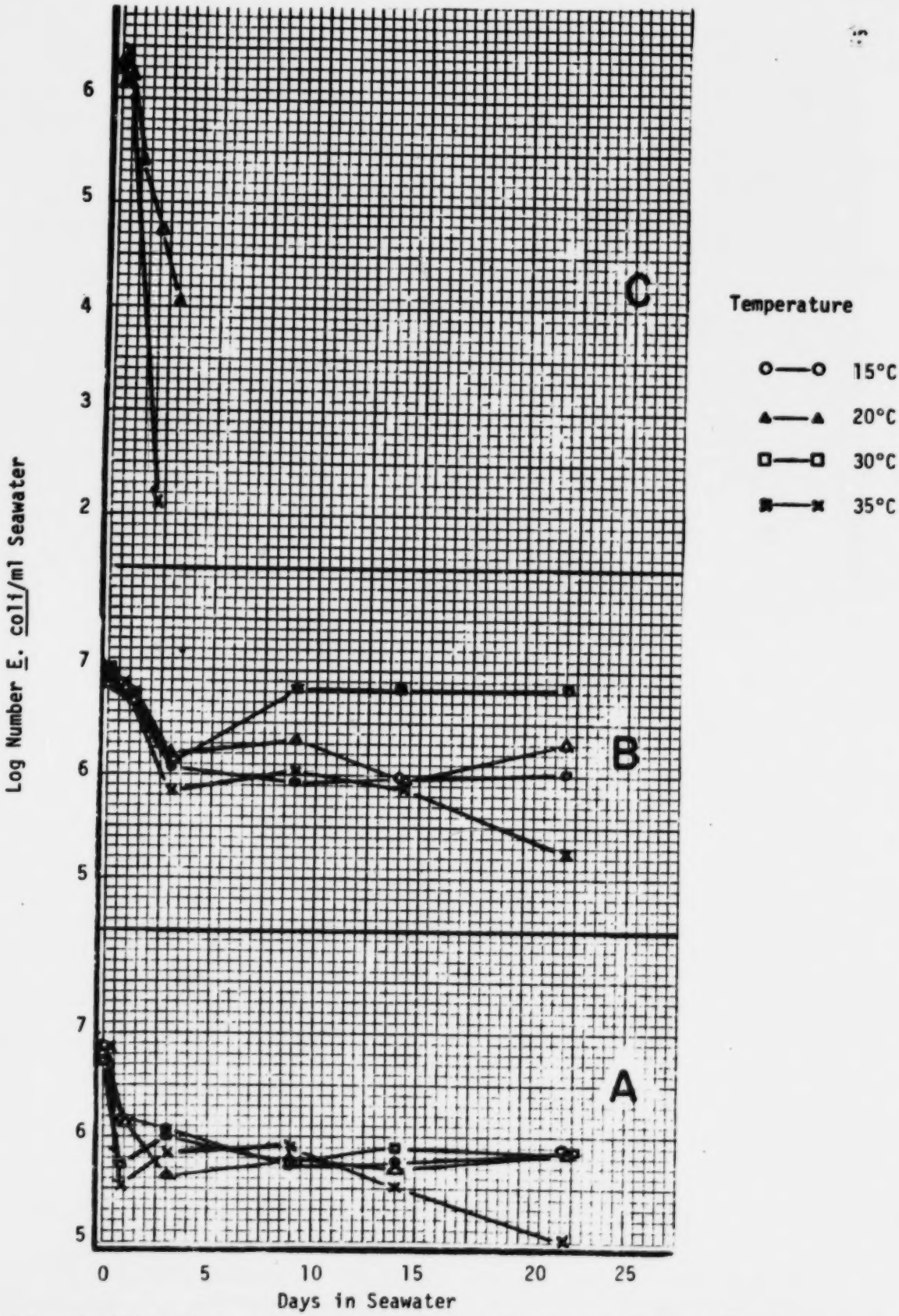

Figure 15. Viability of E. coli at various temperatures in seawater (25 ppt).
(A) Exponential phase bacteria pregrown in M-9 medium at 35°C.
(B) Stationary phase bacteria pregrown in TSB at 35°C.
(C) Exponential phase bacteria pregrown in TSB at 35°C.

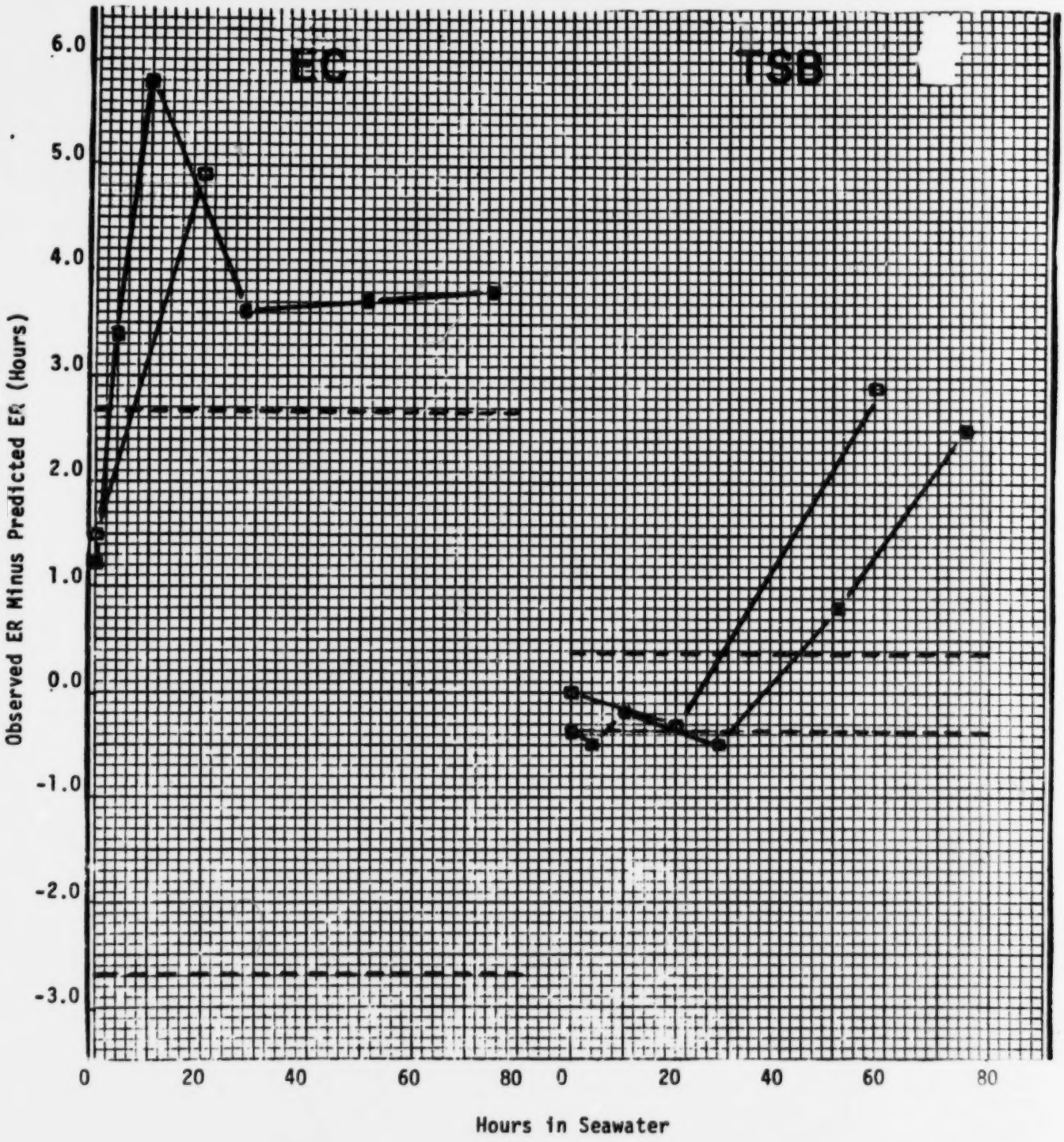

Figure 16. Effect of starvation at 20°C in seawater (25 ppt) on the endpoint response (ER) of E. coli inoculated into either EC or TSB at 44.5°C. Prior to starvation the isolate (urinary tract) was pregrown in TSB at 35°C. Viable counts in both experiments determined using PBS at room temperature. Expt. 1 □——□ and Expt. 2 ■——■.

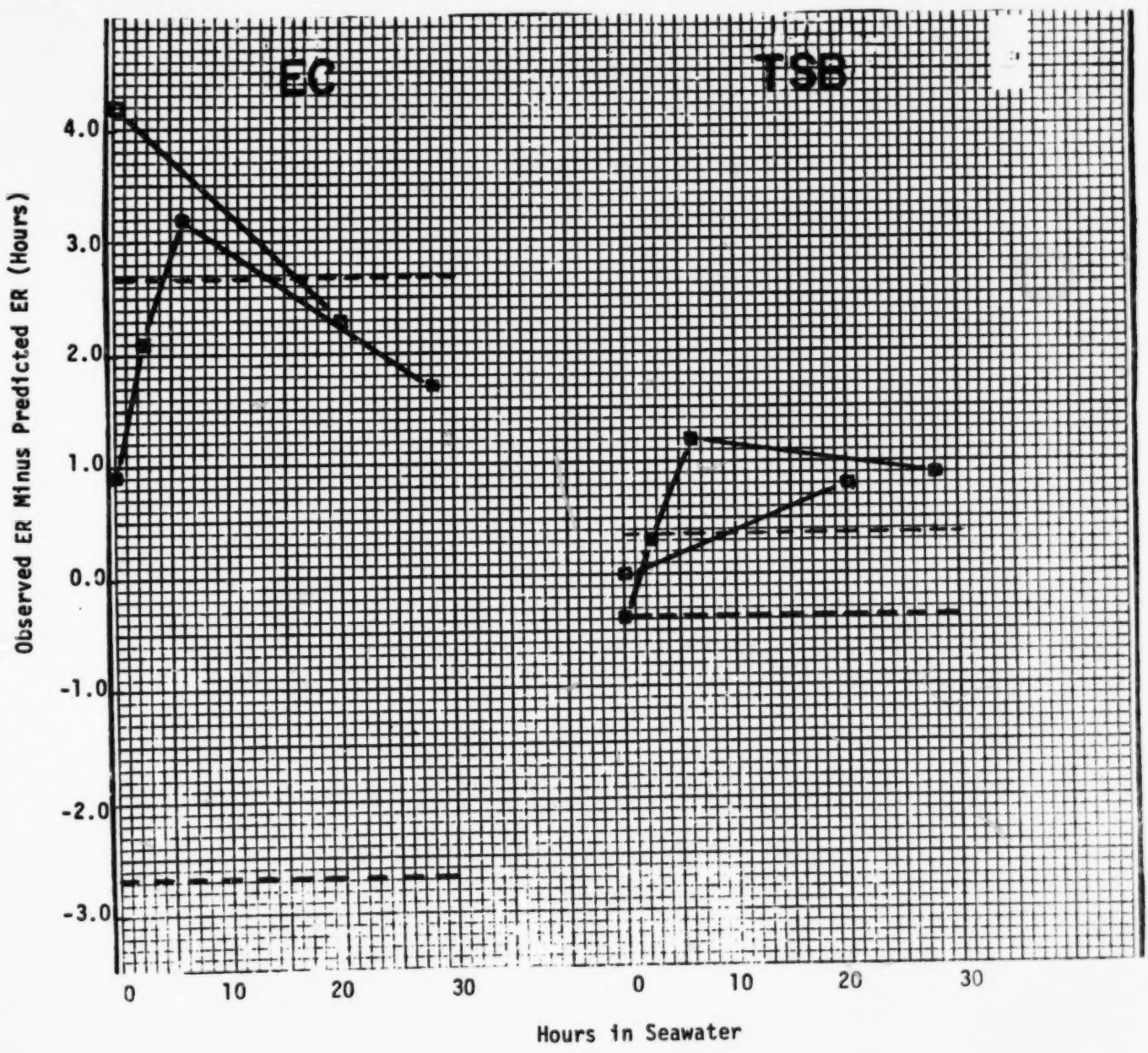

Figure 17. Effect of starvation at 35°C in seawater (25 ppt) on the endpoint response (ER) of E. coli inoculated into either EC or TSB at 44.5°C. Prior to starvation the isolate (urinary tract) was pregrown in TSB at 35°C. Viable counts determined using PBS (□——□) at room temperature or TSB (■——■) at 35°C.

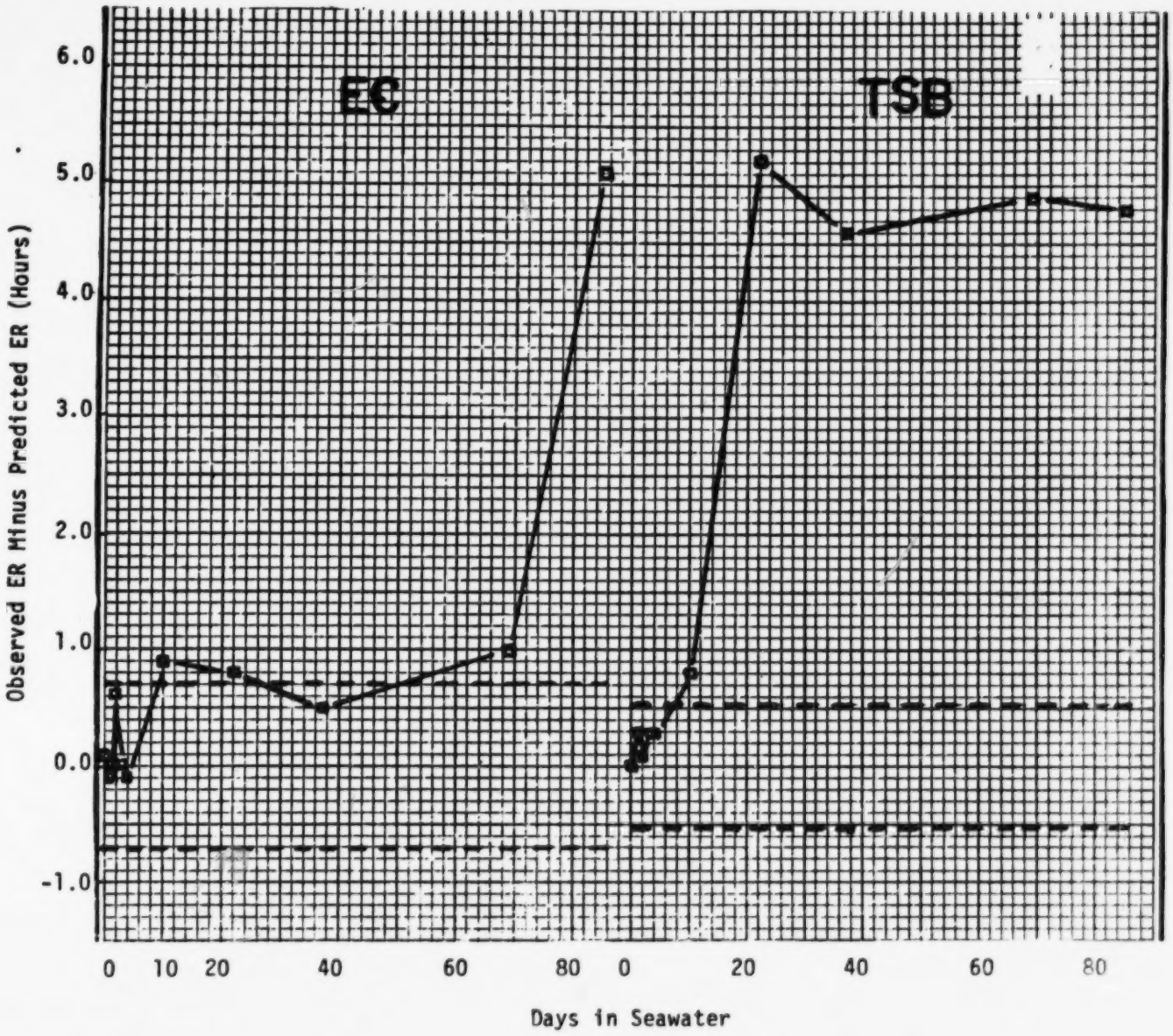

Figure 18. Effect of starvation at 2°C in seawater (25 ppt) on the endpoint response (ER) of E. coli inoculated into either EC or TSB at 44.5°C. Prior to starvation the isolate (feces) was pregrown in M-9 medium at 35°C. Viable counts determined using PBS diluent at room temperature.

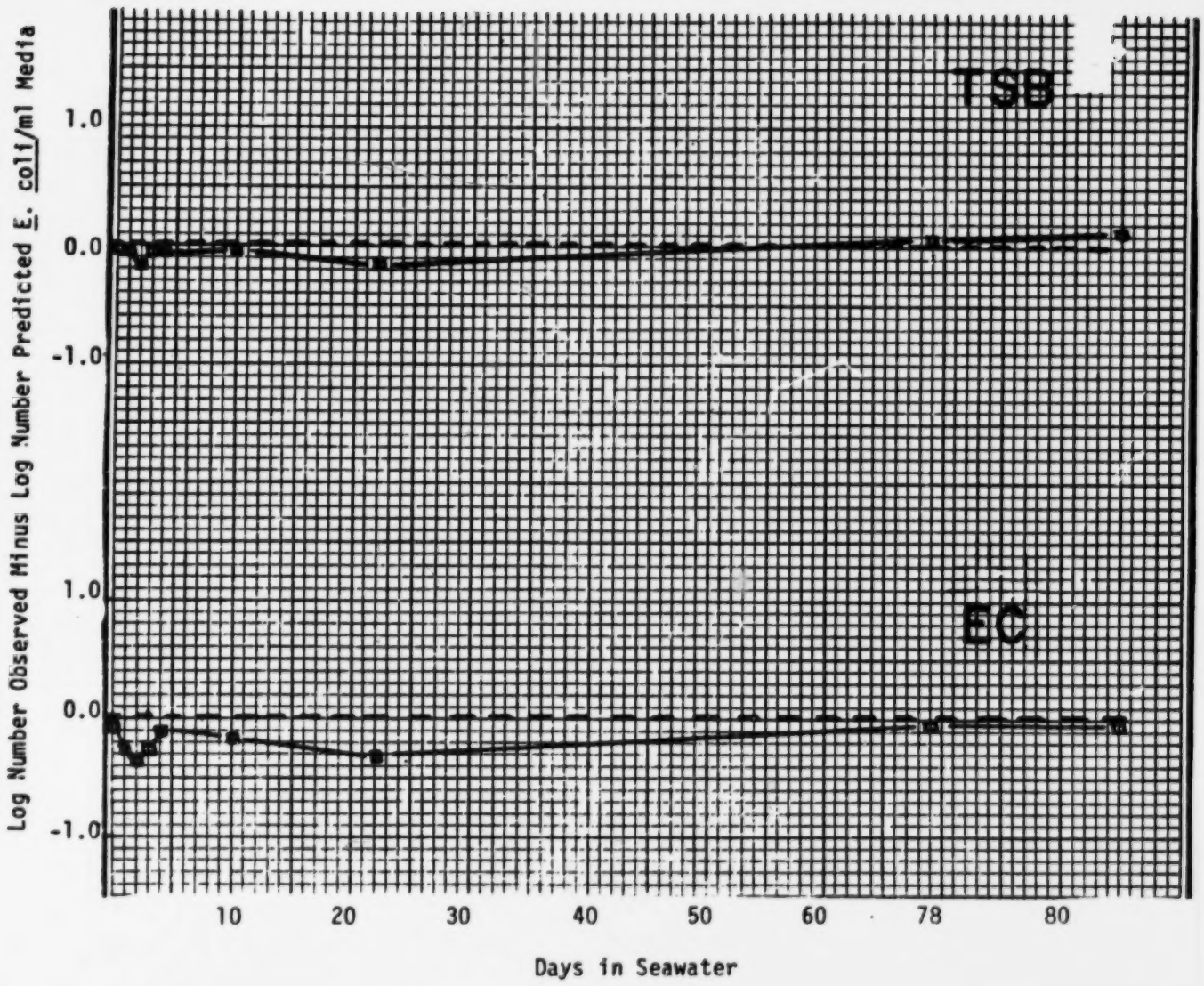

Figure 19. Effect of starvation at 2°C in seawater (25 ppt) on the enumeration of E. coli immediately following a tenfold dilution into either EC or TSB at 44.5°C. Viable counts determined using PBS diluent at room temperature.

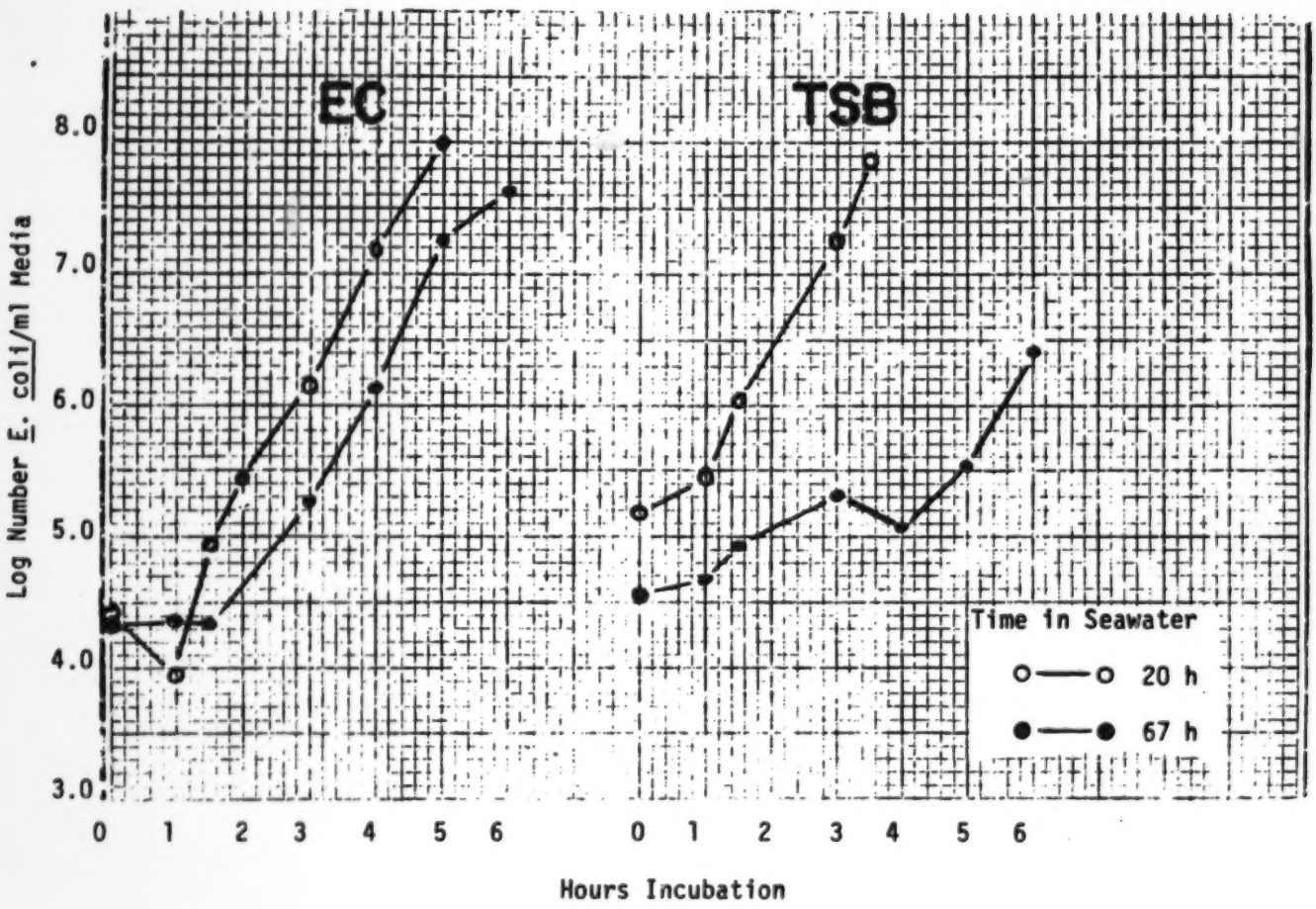

Figure 20. Growth of E. coli in EC and TSB, 44.5°C after 20 and 67 hours starvation at 20°C in seawater (25 ppt). Prior to starvation the isolate (feces) was pregrown in M-9 medium at 35°C.

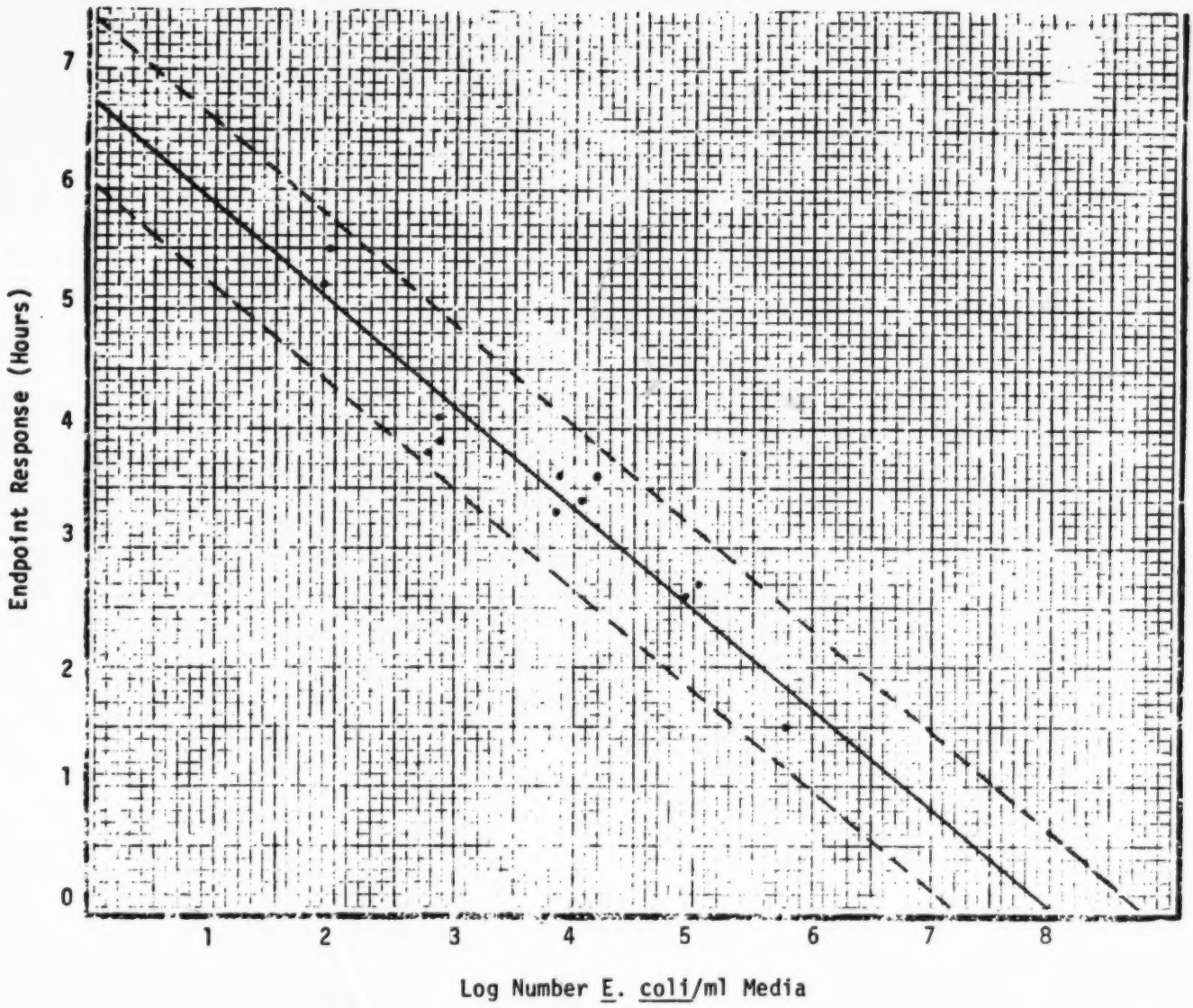

Figure 21. Relationship between inoculum size and the time of endpoint response in half-strength Medium A-1 when inocula were pregrown in TSB at 35°C. A linear least-squares regression calculation gave a correlation coefficient of -0.97 (N = 12) with an intercept of 6.71 and a slope of -0.84. The standard error of estimate at the 95 percent confidence level was ± 0.69.

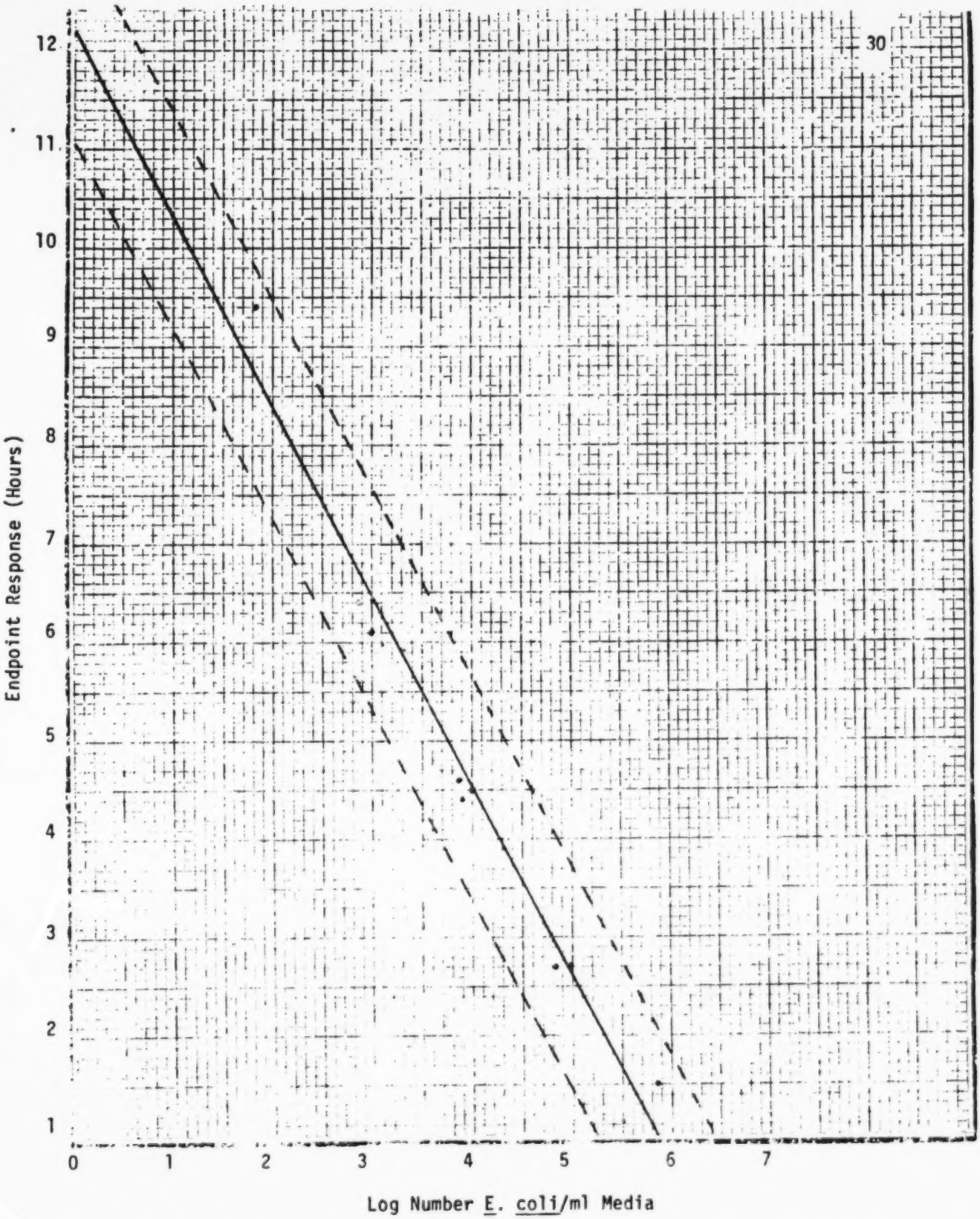

Figure 22. Relationship between inoculum size and the time of endpoint response in half-strength EC at 44.5°C when inocula (urinary tract isolate) were pregrown in TSB at 35°C. A linear least-squares regression calculation gave a correlation coefficient of -0.99 (N = 9) with an intercept of 12.17 and a slope of -1.91. The standard error of estimate at the 95 percent confidence level was ± 1.14.

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15. Supplementary Notes

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16. Abstract

This report presents the results of two research programs to determine the optimum detector design for measuring fecal coliforms in saline waters for operational systems. One program was concerned with the effects of temperature and salinity on endpoint response time, and the other, the interaction between electrode configurations and the test organisms. Test results show that the endpoint response time is related to salinity and seawater temperature; however, these results can be minimized by the correct choice of growth media. Electrode configurations were developed from stainless steel, Parlodion-coated stainless steel and platinum that circumvented problems associated with the commercial redox electrodes.

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